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**Validity of Laboratory Tests
for Predicting Copper Toxicity
in Streams**

Environmental Research Lab -Duluth, Cincinnati, Ohio

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VALIDITY OF LABORATORY TESTS FOR PREDICTING COPPER TOXICITY IN STREAMS



Environmental Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Duluth, Minnesota 55804

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December 1976

VALIDITY OF LABORATORY TESTS FOR PREDICTING
COPPER TOXICITY IN STREAMS

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FOREWORD

The research described in this report was completed to determine if laboratory-based predictions of the effects of a pollutant in a natural waterway are valid. The effort was one of the largest of its kind that we know of and required many years and resources to complete.

While many new questions were raised, the work has served two purposes. First, we found that even though this stream was a complex system, our laboratory-based estimate of effects from the exposure concentration was not far off but somewhat low. Second, the observations made during the study provided a basis for establishing priorities for further research in many areas thereby improving the effectiveness of other programs.

We feel the results of this study provide to EPA confidence that the bioassay data base being generated in our laboratories can be used with confidence as a basis for regulatory action in natural waterways.

Donald I. Mount, Ph.D.
Director
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ABSTRACT

A field study was conducted on Shayler Run, in Clermont County, Ohio, to determine the effects of copper on the stream biota. Copper was added to the stream for 33 months to maintain a concentration of 120 $\mu\text{g/L}$, a concentration that was expected to adversely affect some species of fish and not others. This natural stream received sewage effluent containing a variety of compounds known to affect acute copper toxicity. All but one abundant species of fish in the stream and four of the five most abundant macroinvertebrates were adversely affected by exposure to copper. Direct effects on fish were death, avoidance, and restricted spawning.

To determine the usefulness of laboratory toxicity tests when establishing water quality criteria for an aquatic ecosystem, acute and chronic tests with copper were conducted at the Newtown Fish Toxicology Station and on-site at Shayler Run with stream species and the fathead minnow. The acute toxicity of copper varied widely because of water quality variations in the stream. The chronic tests underestimated the in-stream toxicity by about two times because only the effects of copper on survival, growth, and reproduction were measured but avoidance was not and it was a significant effect in the stream. Agreement between the predictions from laboratory toxicity tests and the observed effect is surprisingly close considering the measurement errors involved.

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We also gratefully acknowledge the excellent cooperation of another Federal agency. The construction and operation of the stream gaging facility by the U.S. Geological Survey was critical to the success of this study. Without their participation and guidance, this field investigation could not have succeeded.

DEDICATION

We dedicate this publication to Jack Geckler whose untimely death at the end of the study was a loss to science as well as to his personal friends.



Without Jack's keen ability to remember what his eyes saw, the study would have lost much of its value. We are thankful Jack was able to complete data collection and the draft report, and we truly appreciate his hard work and diligence in seeing the study through.

We also want to recognize the support and patience of Jack's family that helped him so much, especially his loving wife, Norma. We have tried to make this report a tribute to Jack in the hope that it will help Norma endure her loss and provide Chuck, Linda and Brenda a glimpse of their father's interests and abilities that they can never fully know.

SECTION I

EXECUTIVE SUMMARY

This report describes the results of a study completed by the Newtown Fish Toxicology Station to determine if laboratory estimates of safe concentrations of pollutants are valid in real streams. Copper was used because the laboratory data base was adequate and drinking water supplies would not be jeopardized by the concentration that was expected to adversely affect some fish species and not others.

For 26 months before copper was introduced into the study area, fish, macroinvertebrate, and periphyton populations were sampled to determine baseline data. Spawning and behavioral activities of fish were observed throughout the entire study area. During the 33 months of copper addition, field observations, biannual fish collections, fry collections, and spawning observations were made to evaluate the direct effects of copper on the stream fish populations. Macroinvertebrates were collected to determine the effects of copper on their populations.

Death, avoidance of copper, and restriction of spawning areas were the direct effects observed on the fish resulting in a general decline in fish populations and reduction of food-organism populations. Indirect effects on fish as a result of the effects of copper on the aquatic food chain were not demonstrated.

Copper toxicity varied widely, depending on stream flow stage, season, and water quality. A copper concentration that was not lethal under one stream stage and water-quality condition was rapidly lethal under other circumstances. The tests underestimated the total effect, however, because they did not include avoidance of copper by the fish. Thus, the bioassay data appear to be unconservative when used for estimating safe levels of a toxicant.

SECTION II

CONCLUSIONS

GENERAL

Laboratory-derived data can be used to predict toxic effects in a natural stream situation. In general, the toxicity of copper was underestimated by the laboratory data because avoidance by fish to this metal was not measured in the laboratory tests.

Chronic laboratory toxicity tests with fish can be conducted with natural waters of varying quality, but they are more difficult than tests with water of a more consistent quality.

Indirect effects on fish, as a result of the effects of copper on the aquatic food chain, could not be demonstrated in this study.

Other laboratory tests, such as those based on behavioral responses, may be required in addition to those based on survival, growth, and reproduction, to better predict the effects of a toxicant on a natural ecosystem.

The quality of the field portion of the study was excellent; the data showed the same overall effects of copper on the biota from year to year. Various facets of the study complement each other.

SPECIFIC

On the basis of acute relative sensitivity tests and a fathead minnow chronic test, it was predicted that sunfish would be unaffected and the other fish species would be affected by copper. The predictions were accurate for all fish species except the orangethroat darter.

The order of sensitivity for the various stream species was not consistent from one acute toxicity test to another, but the differences were not great. The data indicate that the order to sensitivity differs with different water quality.

On the basis of chronic toxicity tests, copper was two to three times less toxic to fathead minnows in Shayler Run water, which averaged 270 mg/L hardness (84-356 mg/L.), than in standard (laboratory) water (200 mg/L hardness).

The safe copper concentration for bluntnose minnows in Shayler Run was underestimated by at least two times with the laboratory chronic toxicity tests conducted in Shayler Run water.

The striped shiner, rainbow darter, creek chub, fantail darter, bluntnose minnow, and stoneroller populations in Shayler Run all showed a reduction attributable to copper.

Because of varying water quality, a copper concentration that is near the maximum acceptable toxicant concentration (MATC) may be near the 96-hr LC50 at certain times during the year.

An estimated value for a long-term safe concentration of a toxicant using an application factor approach cannot be based on a single acute toxicity test when dealing with variable water quality.

Detoxifying agents had a major effect on acute copper toxicity, but only minor effects on chronic toxicity, based on values for total copper.

The effect of alkalinity and hardness on acute copper toxicity to fish was not as important as effects of other detoxifying agents.

There is evidence that copper affected the spawning location of green and longear sunfish in Shayler Run. Limited laboratory evidence indicates that, when confined, these fish will spawn at higher copper concentrations than were introduced into the stream.

Copper had a concentration-related effect on the number of eggs produced per female in the laboratory tests.

The maximum acceptable toxicant concentration (MATC) is limited by egg production. Short exposures to copper concentrations above the MATC reduce egg production.

Avoidance of copper by the fish was an important effect in Shayler Run during the first year of the study, but not during succeeding years, probably because the more sensitive individuals had been removed from the populations.

SECTION III

RECOMMENDATIONS

Bioassays should be used to establish water quality standards, when possible, because they provide accurate data to predict effects on aquatic life.

Water quality standards should include the effects of various water quality conditions on toxicity at various stream stages and seasons of the year.

Even short excursions of pollutant concentration above the MATC should not be permitted unless specific data prove they will not be detrimental.

PART A -- INTRODUCTION TO THE STUDY

SECTION IV

INTRODUCTION

Water quality criteria for aquatic life are often developed on the basis of toxicity tests performed in the laboratory, and water quality standards for protection of aquatic life are largely based on these criteria. Some investigators question the validity of the application of laboratory results to a natural situation. Since validity depends on many factors, as discussed by Stephan and Mount (1973), a simple answer to the question is not possible. Extrapolation of results from laboratory tests to different situations is not unique to aquatic toxicology, only the specific circumstances are different.

The usefulness of laboratory aquatic toxicity tests can be studied with artificial streams, such as planned by Merna and Eisele (1973). Although artificial streams and model ecosystems are elaborate laboratory toxicity tests, they do not necessarily accurately simulate natural situations. Theoretically, the best, but practically the most difficult, way is to compare the results of laboratory exposures with the results of field exposures in a natural situation, as done by Sprague and Drury (1969). In this way the effects of natural stresses, such as changes in temperature, flow, water quality, species competition, affects on the food web, and behavioral reactions, can be taken into account. Studies in natural situations are more likely to produce qualitative, circumstantial evidence rather than unequivocal, quantitative results. Slightly modified natural situations, such as those used in this study, are designed to produce quantitative cause-and-effect data under real-life conditions. Many aspects of the field portion of this study were based on experiences of Larimore et al. (1959).

The major purpose of this study was to evaluate the usefulness of laboratory toxicity tests in predicting water quality criteria for protection of aquatic life. The study was designed so that the results from standard laboratory and streamside laboratory chronic tests could be compared with results of long-term stream exposure to a toxicant. The streamside chronic tests varied from the standard laboratory chronic test in that water quality, temperature, and photoperiod were not controlled. The streamside tests were conducted on site, in a temporary building, using control and exposure waters and mixtures of the two. Several resident and one non-resident fish species were tested during the study.

Although the study was not designed to obtain information concerning the validity of the application factor hypothesis (Mount and Stephan, 1967), information relative to it was gained from this study. Effects other than those studied in the chronic tests could occur in the field exposure, so emphasis was placed on stream observations. This single study cannot answer all questions, but it is a major contribution towards the validation of the use of laboratory results in establishing water quality criteria for protection of aquatic life.

Stream selection criteria were: (1) adequate stream flow, (2) little chance of uncontrolled pollution, (3) limited public access, (4) no direct public use of water in the stream, (5) presence of riffles and pools, (6) proximity to the Newtown Fish Toxicology Station in Cincinnati, Ohio, and (7) diverse aquatic flora and fauna. Approval by The State of Ohio Department of Health, the Ohio Department of Natural Resources, and landowners was also needed. Shayler Run, near Cincinnati, Ohio, was chosen and met most of the requirements, except that at times heavy runoff was known to cause excessive flooding and a small domestic sewage treatment plant (extended aeration) was located 4.4 km upstream from the test site.

Copper was chosen as the toxicant for this study for the following reasons: (1) both chronic and acute laboratory tests had been performed successfully with fish; (2) exposure concentration could be rapidly measured; (3) the stream exposure concentration would be less than 1.0 ppm, the maximum recommended for drinking water by the U. S. Environmental Protection Agency, thereby posing no hazard to nearby wells; (4) a sufficient range in sensitivity was exhibited by the stream species; (5) it is a common persistent pollutant; and (6) it does not markedly bioconcentrate.

Chronic tests with fathead minnows and copper had been conducted with a hard water of 200 ppm hardness of CaCO_3 (Mount, 1968) and a soft water of 30 ppm hardness (Mount and Stephan, 1969). Because of the higher hardness and alkalinity and the presence of detoxifying agents from the sewage treatment plant, the maximum acceptable toxicant concentration (MATC) of copper for fathead minnows in Shayler Run water was predicted to be about 75 $\mu\text{g}/\text{L}$. The MATC is defined as the highest toxicant concentration that has no adverse effect on survival, growth, and reproduction of the test organism. Preliminary acute mortality tests indicated that most of the important fish species in Shayler Run were about as sensitive to copper as were fathead minnows except sunfish, which were much less sensitive. We therefore concluded that a concentration of 120 μg copper/ L probably would adversely affect the more sensitive species, but not the sunfish. Higher concentrations would be lethal to some stream species when hardness, alkalinity, and the concentration of detoxifying materials were lower because of rain. Thus, 120 $\mu\text{g}/\text{L}$ seemed to be a concentration of copper that would be adverse to some species, but not to others, and would allow us to evaluate its effect on sensitive and resistant species of fish.

The field portion of the study lasted for 4 years and 9 months. This time span was divided into two periods: the pre-exposure period lasted from December 1967 to February 16, 1970; the exposure period lasted from February 16, 1970, to October 31, 1972. The chronic toxicity studies continued until January 18, 1973. Limited stream sampling for chemical parameters continued through May, 1973.

Part A of this report deals with the rationale of the project, description of the field study site, physical alterations made on this site, toxicant metering system, general water quality, and stream copper concentrations over the period of exposure. Part B reports findings relative to the effects of copper on stream fish and benthic communities and the analyses of stream fish stomach contents. Part C presents the results of laboratory tests done in conjunction with the field study before and during the stream exposure. Part D is a discussion that relates the field studies to the laboratory studies.

SECTION V

DESCRIPTION OF THE STUDY AREA

Shayler Run, located in Clermont County, Ohio, is a tributary of the East Fork of the Little Miami River. The use of the watershed is predominantly urban, but areas adjacent to the study portion of the stream are wooded or used for grazing (Figure 1). The laboratory site on the stream is latitude $39^{\circ} 06' 46''$, longitude $84^{\circ} 13' 24''$, and is 3.5 km from the mouth (Figure 2). The drainage area is 30.58 km^2 , and the stream gradient in the study stretch is 26.8 m/km. The stream consists of a series of limestone riffles and shallow pools, with a minimal amount of sedimentation. At normal stream flow the pools are 3-9 m wide and 1-3.5 m deep. Riffles comprise more than 50% of the stream length at normal stream flow and are 3-12 m wide.

The only continuous flow (1890 kiloliters/day) originates from a small domestic sewage treatment plant that, during low flow periods, may contribute as much as 90% of the stream discharge. Data from macroinvertebrate collections obtained between April 1969 and February 1970 indicate that the effluent from the sewage treatment plant had minimal, if any, effect on the aquatic biota in the section of stream considered in this report. Flow from Arrowhead Lake is only intermittent.

The U. S. Geological Survey designed, constructed, installed, and maintained a stream gaging system approximately 4.4 km downstream from the waste treatment plant outfall (Figure 3). This system consisted of a gaging pool and V-notch weir (Figure 4), bubble gage (water-stage servo-manometer with gas purge system), and a Stevens water-level recorder. The gaging system was completely installed by July 1968 and functioned accurately throughout the study period.

The study area was divided at the V-notch weir into an upstream control area (487 m long) and a downstream exposure area (902 m long). Copper was added to the stream at the weir, which is designated as the zero point for both elevation and distance. The remaining 2.7 km of the stream below the exposure area to the confluence with the East Fork of the Little Miami River was called the recovery area. This stretch of stream was similar to the experimental area, but of lower gradient. In this area the copper concentration was less than that in the exposure area and decreased with distance downstream. Limited sampling in this area provided some information on repopulation of the stream by macroinvertebrates that were adversely affected by copper in the exposure area.

Fish weirs were constructed to prevent upstream migration and to capture fish moving downstream from both the control and exposure areas (Figure 3 and 5). They were constructed of concrete, steel, and stainless steel wire screen and were placed at the upper and lower ends of the 0.8-km exposure section. These weirs were a modification of a Wolf-type fish-counting fence described by Clay (1961), but were more permanent structures.



Figure 1. Aerial view of Shayler Run study area, looking downstream.

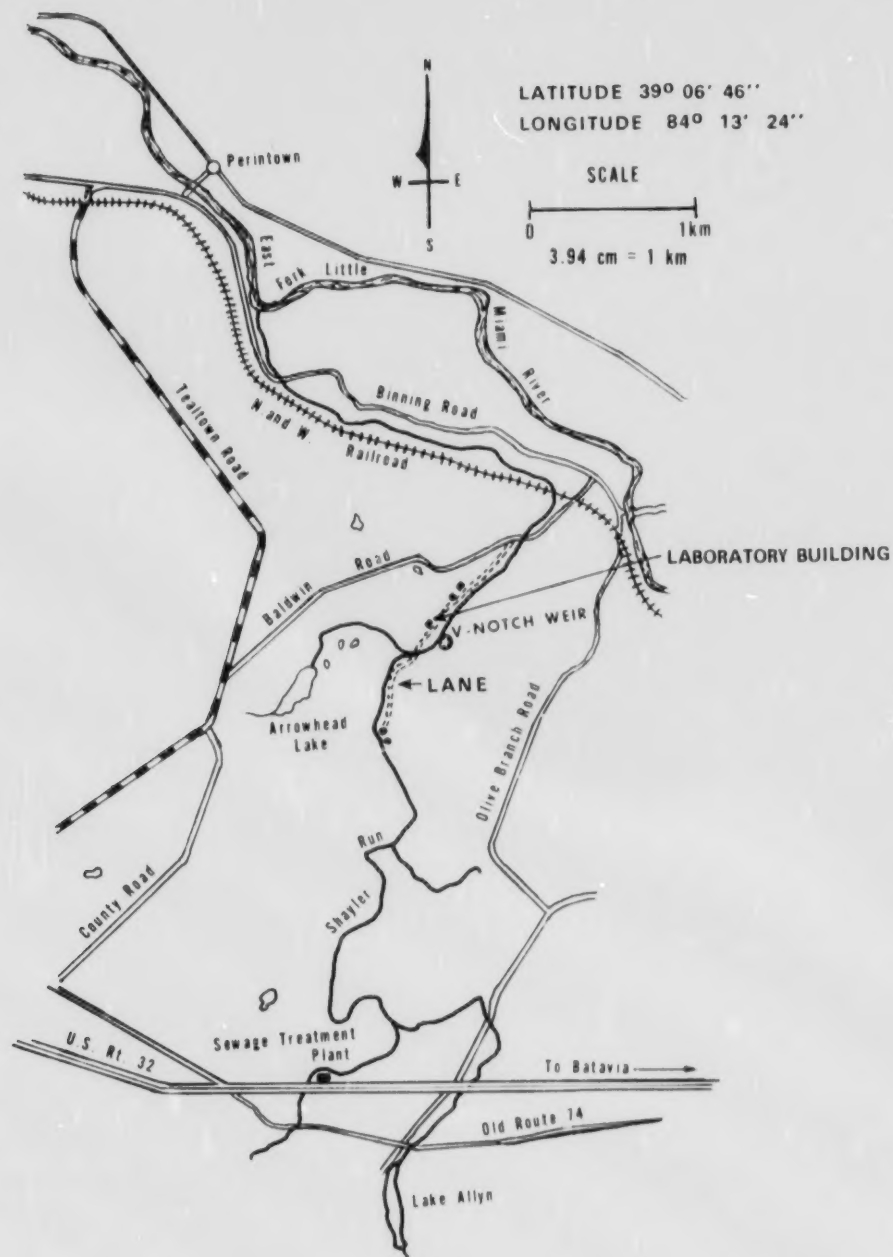


Figure 2. Location map of Shayler Run.

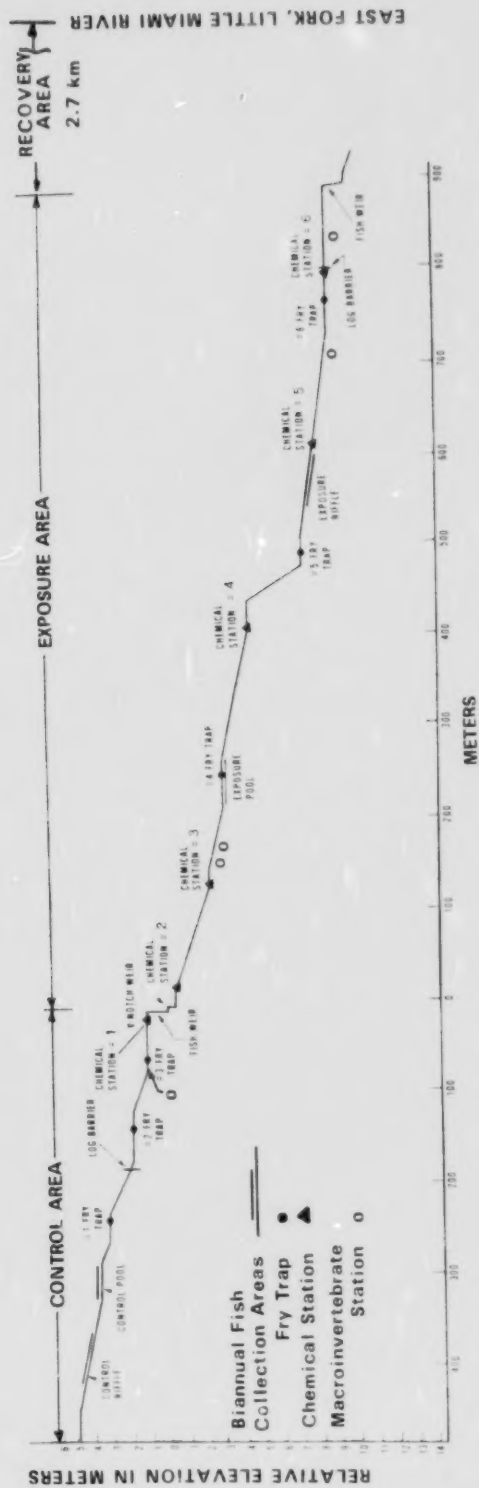


Figure 3. Sampling stations and gradient for Shayler Run.

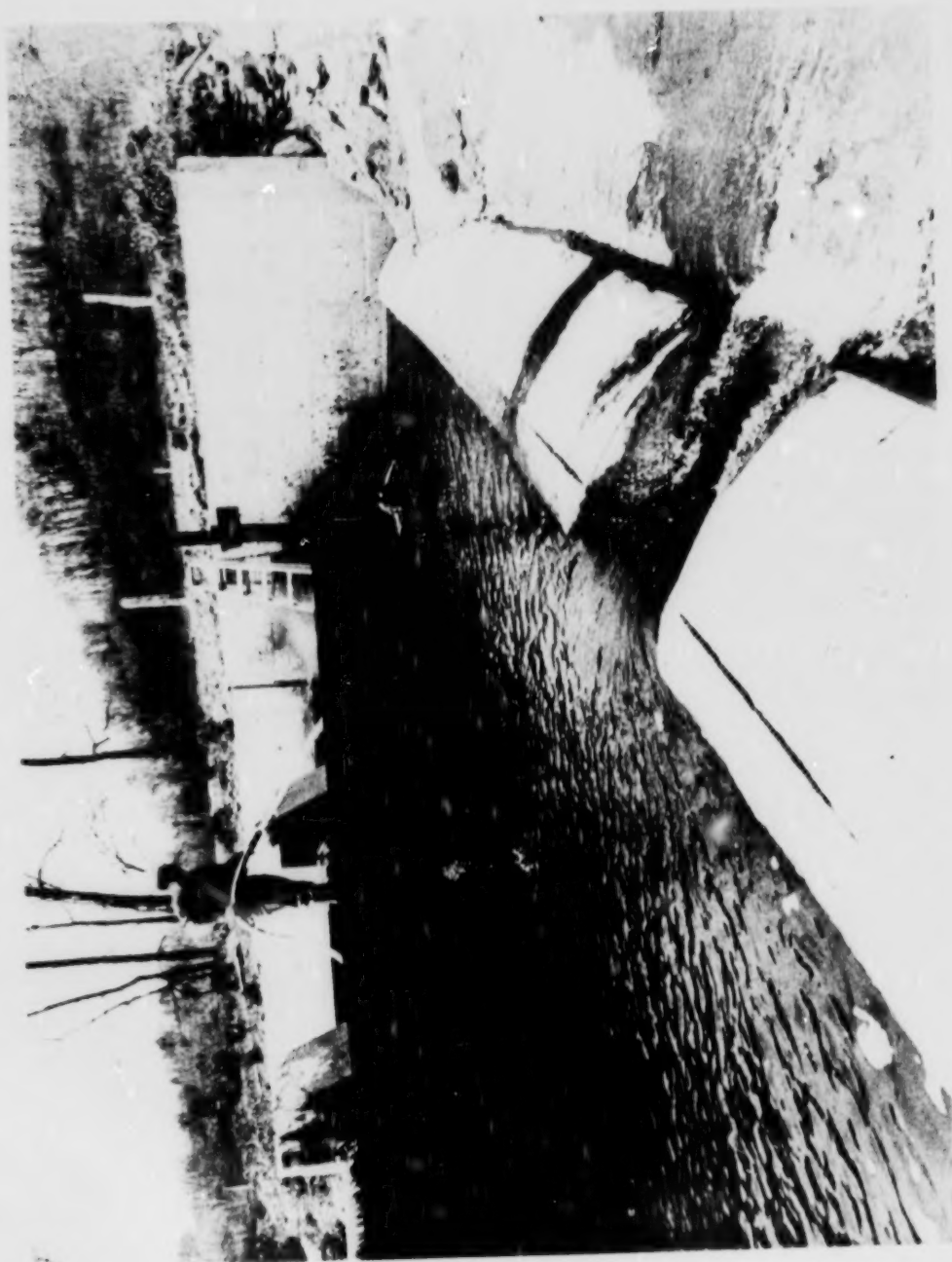


Figure 4. V-notch gaging weir.

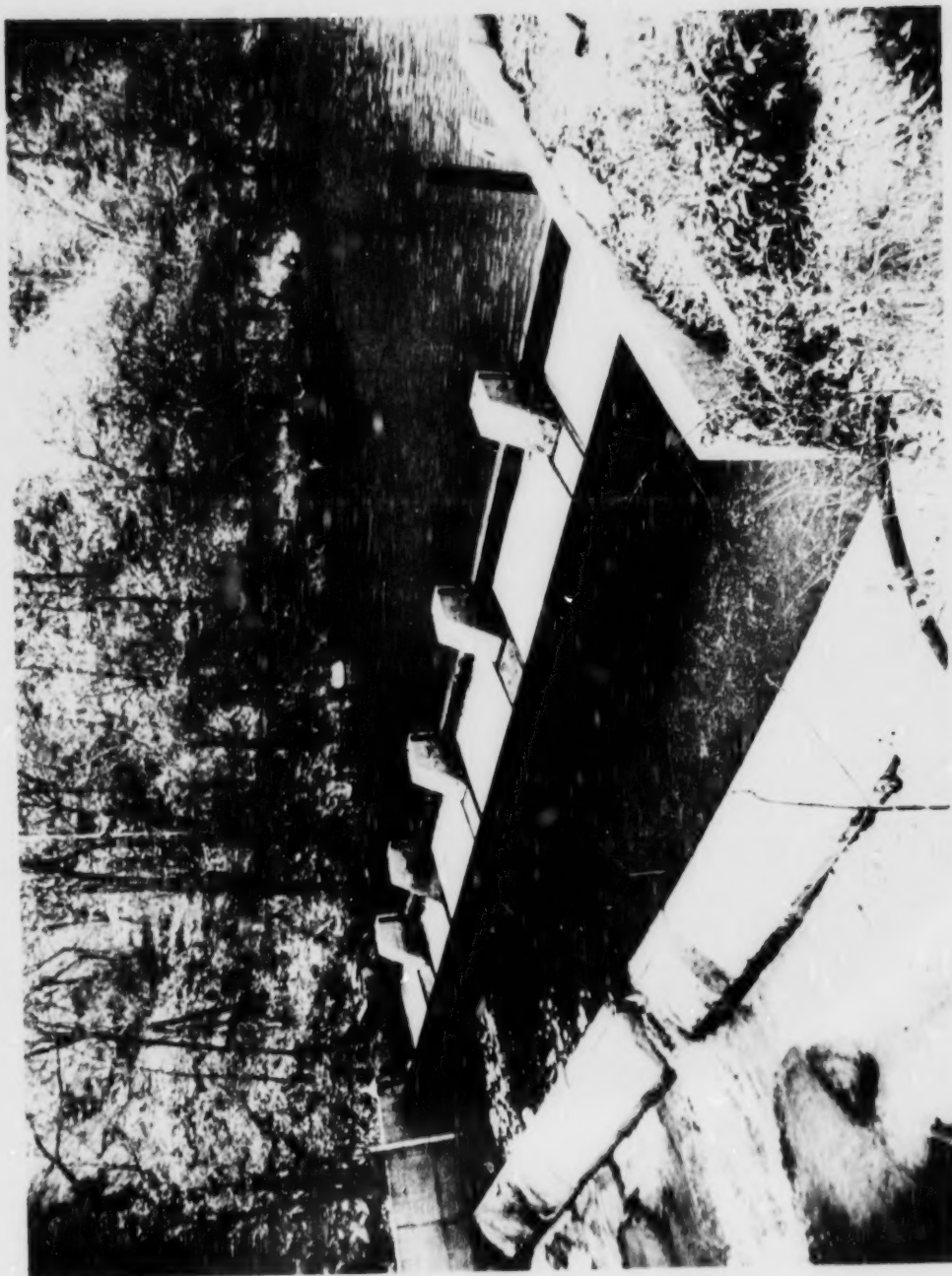


Figure 5. Control weir screens and V-notch gaging weir

When stream flow exceeded $1.7 \text{ m}^3/\text{sec}$, the screen could not be kept clean, and water flowed over the top allowing unmeasured fish passage. From March through November a fine stainless steel screen with openings of 0.5 mm was used to capture both fish fry and macroinvertebrates. From November through February a coarser screen with 2.2-mm openings was installed in place of the finer screen to reduce maintenance time. This coarser screen captured young-of-the-year fish.

Log barriers were constructed approximately 120 m upstream from both control and exposure fish weirs to protect the stainless steel screens from damage from large floating debris during heavy runoff. These barriers (Figures 6 and 7) were constructed of 15.2-cm steel casings driven into the stream bed and filled with reinforcing rods and concrete. Three lengths of 2.5-cm steel cable were then attached to the castings and anchored to a concrete deadman on each side of the stream. Vertical pieces of cable were placed at equal distances on three horizontal cables. The barriers were placed at a 45-degree angle to the stream flow so that debris would be shunted to one side. The log barriers efficiently protected the fish weirs.

The locations of the sampling stations for chemical analysis and for fish, macroinvertebrate, and periphyton collections, are shown in Figure 3. Station 1 was located just upstream from the V-notch weir, and station 2 was 15 m downstream from the V-notch weir. The Baldwin Road bridge was 850 m downstream from the V-notch weir, near the downstream log barrier. The other chemical sampling stations, 3, 4, 5, and 6, were located 130, 410, 620, and 795 m downstream from the V-notch weir, respectively. The control riffle and control pool stations were 350 and 275 m, respectively, upstream from the V-notch weir; the exposure pool and exposure riffle were 200 and 525 m, respectively, downstream from the V-notch weir. Fry traps were placed 50 (#3), 125 (#2), and 225 m (#1) above and 250 (#4), 475 (#5), and 775 m (#6) below the V-notch weir. Basket samplers for collection of macroinvertebrates were located as follows: (1) control area--midstream at fry trap 3; (2) upper exposure area--30 m downstream from chemical station 3; (3) lower exposure area--20 m downstream from chemical station 6; and (4) recovery area--2 km downstream from V-notch weir. The areas for sampling natural substrates for macroinvertebrates were the exposure riffles, 70 m downstream from chemical station 3 and 100 m downstream from chemical station 5, and in a recovery riffle, 2.2 km downstream from the V-notch weir (not shown on Figure 3).

Periphyton samples were collected from natural and artificial substrates located in the control riffle and control pool, and from station 4 (pool) and the exposure riffle in the exposure area.

Stream-flow recording began on August 1, 1968, after completion of the gaging pool and weir and the calibration of the system. Charts and data were analyzed by the U. S. Geological Survey and recorded in Water Resources Data for Ohio, Part 1, for 1970, 1971, and 1972 (Appendix Table 1).

Daily mean flow was 50% lower during the pre-exposure period than during the exposure period. After periods of heavy runoff the stream flow decreased to $0.25 \text{ m}^3/\text{sec}$ within 24 hr after the crest had passed the V-notch weir.



Figure 6. Upstream log barrier.

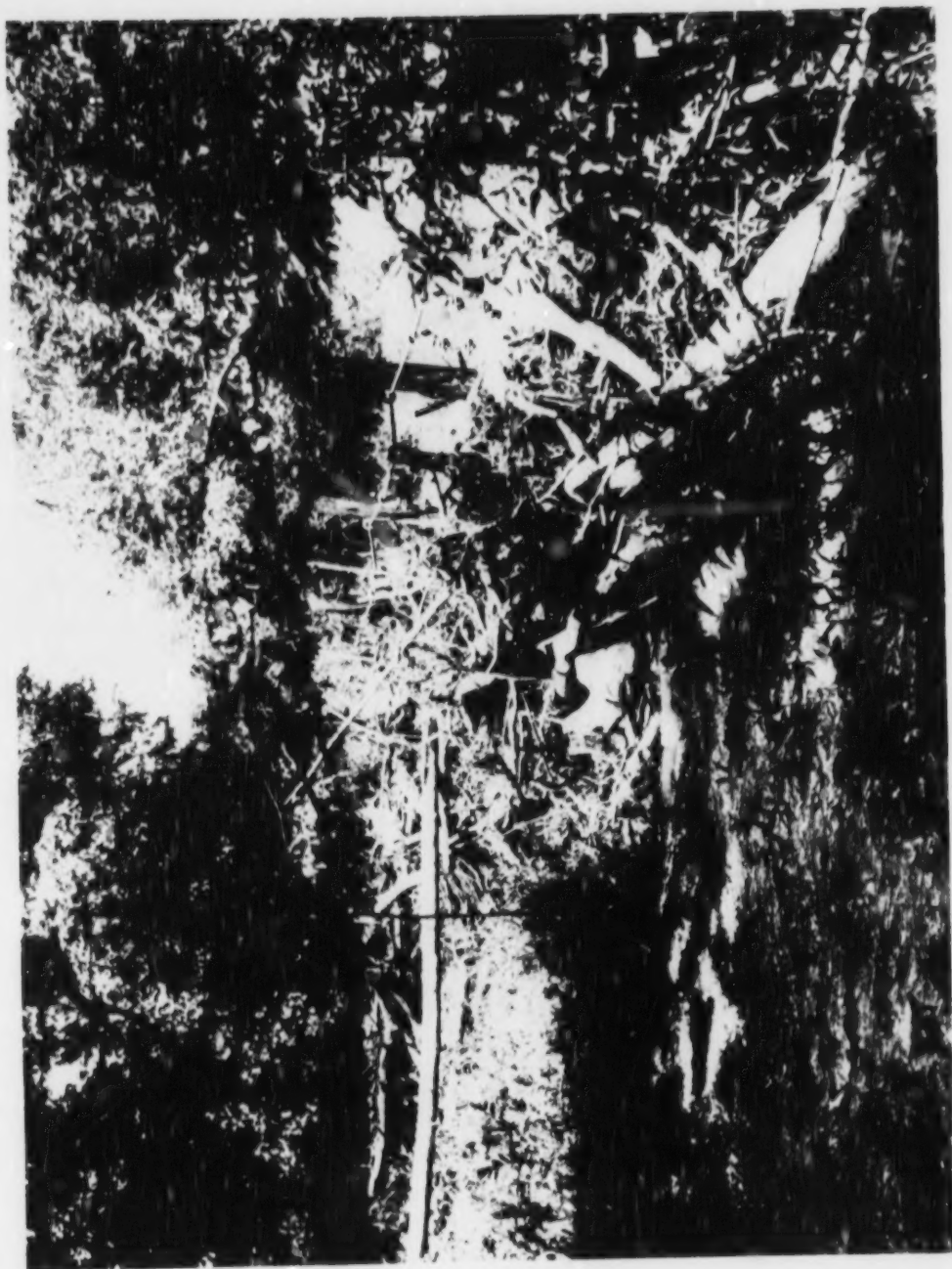


Figure 7. Upstream log barrier covered with debris and logs.

SECTION VI

STREAMSIDE LABORATORY FACILITIES

A 12.2- by 2.4-m mobile laboratory trailer and a 6.1- by 9.1-m sheet metal pole building with an attached 12.2- by 3.0-m outside screened porch constituted the laboratory facilities. These were located adjacent to the stream, beside the V-notch weir. The mobile laboratory trailer was used for routine chemical analyses, for processing some of the biological samples, and as an office. The fish holding tanks and toxicity testing systems were located in the pole building and its attached porch (Figure 8). This building also housed the copper stock-supply reservoir. The pole building was the only wet laboratory area during the first year of the test.

Two 314 L./min self-priming centrifugal cast iron pumps continuously supplied water for the test systems. One pump supplied control water from just above the V-notch weir. The other pump supplied exposure water, nominally dosed at 120 $\mu\text{g/L}$ copper, from station 3, 130 m below the V-notch weir where thorough mixing had taken place. The water from each pump was pumped through polyethylene pipes to separate manifolds in the wet laboratory and was distributed to the flow-through test systems, water baths, and holding tanks. The pumps had 6.3-mm intake strainers and flow switches to turn them off automatically when flow was interrupted, thus preventing damage to the pump heads.

During the first year and a half of the study a constant pressure regulator and strainer assembly was used to maintain a constant flow for the test systems. For the last year and a half, water was pumped to small-volume headboxes, located in the rafters of the wet laboratory, to maintain a constant pressure.

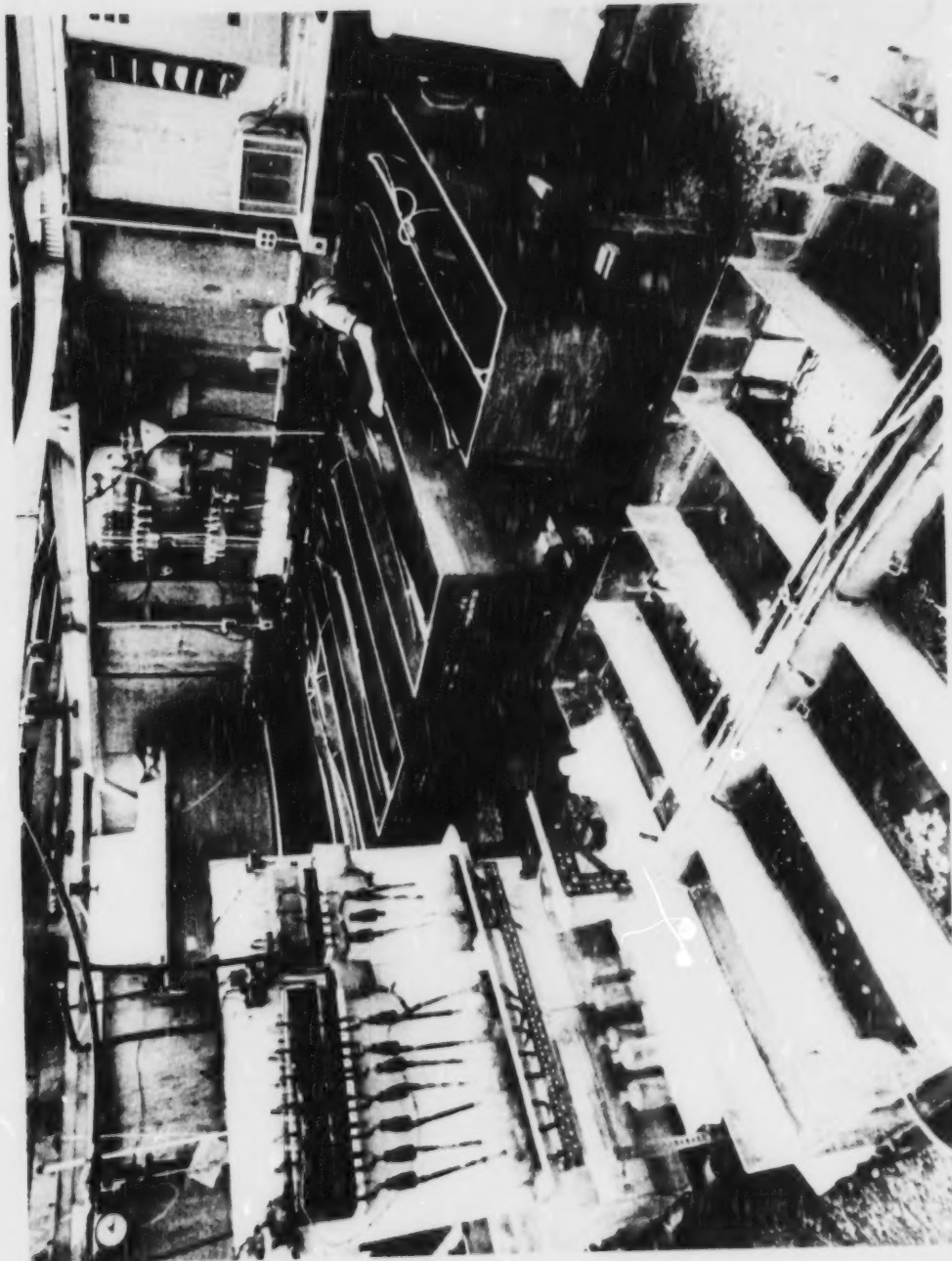


Figure 8 Interior of pole building wet laboratory showing chronic test setup.

SECTION VII

TOXICANT-METERING SYSTEM

Initial addition of copper to the stream, beginning February 16, 1970, was done with a gear-type pump having a manually controlled pumping rate. Staff personnel were required to adjust the pumping rate, as stream flow varied, to maintain the desired copper concentration in the stream. Use of an automatic toxicant-metering system began on May 18, 1970, and was continued for the remainder of the study. This system was designed so that, at stream discharges of $0.25 \text{ m}^3/\text{sec}$ and below, copper sulfate solution would be metered to the stream to maintain a nominal concentration of $120 \text{ } \mu\text{g}/\text{L}$ at station 3, the point of thorough mixing (Figure 9). The stream-gaging apparatus was used to control the pumping rate. The stream was not dosed at flows above $0.25 \text{ m}^3/\text{sec}$ because of the large quantity of copper sulfate required.

Technical grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was dissolved in deionized water in a 2,000-L fiber glass tank. Aeration was used to mix the solution that contained $3.06 \text{ g Cu}/\text{L}$. The stock solution was then automatically siphoned to a 2,000-L feed tank for the pump.

During the exposure period, February 16, 1970, to October 31, 1972, copper was added 75% of the time. The dosing pattern varied from month to month (Table 1). For any given month over the 33-month period, the amount of time that dosing occurred varied considerably since no toxicant was added at flows greater than $0.25 \text{ m}^3/\text{sec}$.

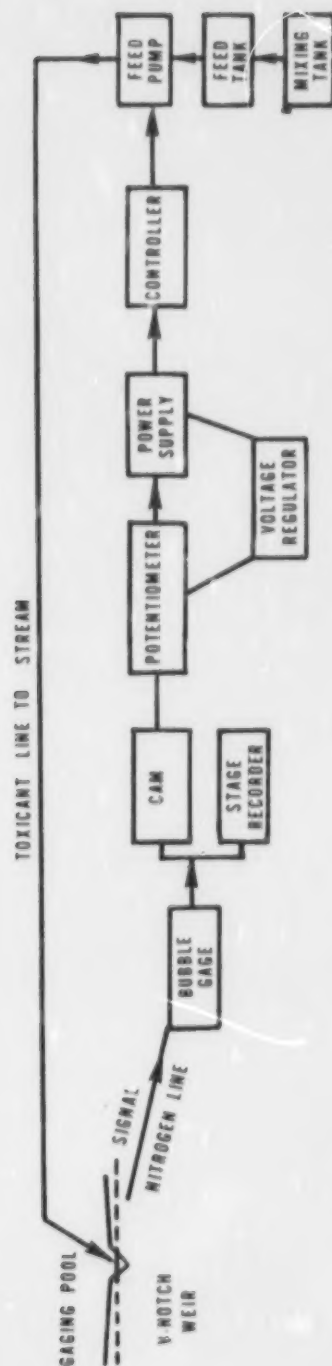


Figure 9. Schematic of toxicant-delivery system.

TABLE 1. PERCENTAGE OF TIME THAT COPPER WAS ADDED TO SHAYLER RUN DURING THE EXPOSURE PERIOD

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.	Average
1970	-	95	57	57	90	96	98	84	100	98	91	73	85
1971	83	21	46	97	88	98	87	95	74	100	97	59	79
1972	62	36	17	30	58	93	96	64	85	90	-	-	63

SECTION VIII

WATER QUALITY

TEMPERATURE

Temperatures for Shayler Run during the 3 months of copper addition were recorded continuously from the gaging pool. The minimum and maximum daily temperatures normally occurred at 6 a.m. and 6 p.m., respectively. Data were taken from daily records at four different times, 6 a.m., 12 N, 6 p.m., and 12 M, for calculating the daily means. Monthly means were calculated from the daily means (Appendix Table 2). Temperature regimes for the 3 years were similar.

CHEMICAL

Five different sets of measurements were performed periodically to determine the chemical characteristics of Shayler Run water collected at station 1, which is located upstream from the point of copper addition. Analyses for pH, alkalinity, hardness, and dissolved oxygen constituted set 1 and were performed on-site (Appendix Tables 3, 4, 5, and 6).

Set 2 measurements were performed at the Newtown Fish Toxicology Station on samples generally less than 4 hr old. Analyses were pH, alkalinity, hardness, specific conductivity (SC), total solids (TS), dissolved solids (DS), calcium (Ca), magnesium (Mg), orthophosphate-phosphorus (OP-P), and total phosphorus (TP) (Appendix Table 7). Recommended methods were used (U. S. Federal Water Pollution Control Administration, 1969), except that dissolved solids were defined as those that passed through a 0.45- μ membrane filter.

Set 3 measurements were performed on a portion of each sample used in set 2. Analyses were potassium (K), sodium (Na), chloride (CHD), nitrate-nitrogen ($\text{NO}_3\text{-N}$), nitrite-nitrogen ($\text{NO}_2\text{-N}$), ammonia-nitrogen ($\text{NH}_3\text{-N}$), organic-nitrogen (ORG-N), total Kjeldahl-nitrogen (TKN), total phosphorus (TP), total organic carbon (TOC), calcium (Ca), and magnesium (Mg) (Appendix Table 8).

These analyses were performed under the supervision of Robert T. Williams by the Waste Identification and Analysis Section of the Waste Water Research Division of the Municipal Environmental Research Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio, (formerly known as the Advanced Waste Treatment Research Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio), on water samples from Shayler Run that were generally less than 8 hr old. Recommended methods were used (U. S. Federal Water Pollution Control Administration, 1969), except that chloride (CHD) was measured with an automatic titrator and silver nitrate.

Thirteen measurements, constituting set 4, were made for pesticides. These were DDT, DDE, DDD, chlordane, dieldrin, endrin, heptachlor, heptachlor epoxide, aldrin, BHD, lindane, endosulfan, toxaphene, and methoxychlor. Values for these pesticides were generally less than detection limits, which were between 50 and 1000 ng/l. for toxaphene and between 3 and 36 ng/l. for the others. These measurements were performed under the supervision of James J. Lichtenberg by the Pesticides Identification Group of the Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio, (formerly known as the Analytical Quality Control Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio), using recommended methods (U. S. Environmental Protection Agency, 1971). Four samples were also analyzed for malathion, parathion, methyl parathion, trithion, methyl trithion, fenthion, ethion, DEF, and dimethoate. The concentrations were always less than the detection limits, which were between 10 and 50 ng/l. Fifteen samples were analyzed for trace metals (Appendix Table 9). These constituted set 5 measurements and were performed under the supervision of John F. Kopp of the Metals Analyses Group of the Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio, (formerly known as the Analytical Quality Control Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio), using an emission spectro-chemical method (American Society for Testing and Materials, 1971).

SECTION IX

STREAM-COPPER ANALYSIS

The background concentration of copper (Cu) in Shayler Run water was measured with a solvent extraction and atomic absorption spectrophotometric procedure like that used by Brungs *et al.* (1973). Samples were acidified with 3 drops of concentrated nitric acid per 100 ml. One to three hundred milliliters were poured into a tared separatory funnel and weighed, and 2 ml concentrated nitric acid were added per 100 ml. The sample was then extracted three times with 25 ml of a solution made by dissolving 4 g diethylammonium diethyldithiocarbamate (Stary, 1964) in 1 gal distilled-in-glass chloroform, shaking the extractions for 2 min, 30 sec, and 30 sec, respectively. The extracts and any interfacial cuff were collected in a beaker and (a) evaporated to dryness on a steam bath, or (b) 1 ml of bromine or peracetic acid solution was added to destroy the volatile copper complex before the solution was evaporated to dryness on a hot plate. The peracetic acid solution was prepared fresh daily by mixing 2 ml of 30% hydrogen peroxide with 10 ml of glacial acetic acid (Patchett and Batchelder, 1960). The sides of the beaker were washed down with concentrated nitric acid, and the solution was evaporated to dryness. The residue was dissolved in 0.15% concentrated nitric acid and analyzed on a Perkin-Elmer model 303 atomic absorption spectrophotometer with an air-acetylene flame and a Belling burner. Standards prepared in the laboratory by dissolving reagent-grade copper sulfate in 0.15% concentrated nitric acid in distilled water were checked against purchased copper standard solutions.

Between January 21, 1969, and November 2, 1972, 100 recoveries, using 300 ml Shayler Run water spiked with 10-20 $\mu\text{g Cu/L.}$, averaged 95.2% with a standard deviation of 3.5% after three values of 132, 60, and 58% were discarded as outliers. Sixty-two recoveries, using 100 ml Shayler Run water spiked with 30-60 $\mu\text{g Cu/L.}$, averaged 98.1% with a standard deviation of 4.4% after one value of 75% was discarded as an outlier. The outliers were all more than four standard deviations from the respective means.

Between March 10, 1970, and December 3, 1971, 52 samples of undosed Shayler Run water containing between 1 and 17 $\mu\text{g Cu/L.}$ (average 6) were analyzed in duplicate and seven in triplicate. The ratio of the higher result to the lower in each set averaged 1.08 and ranged from 1.00 to 1.40 after two values of 2.07 and 2.54 were discarded as outliers. The coefficient of variation (relative standard deviation) of the method calculated from the replicates averaged 7% and ranged from 0% to 23% after two values of 49% and 61% were discarded as outliers, when Cochran's test for homogeneity of variances was used (Guenther, 1964).

Between October 28, 1970, and August 19, 1971, 50 samples of dosed Shayler Run water containing between 15 and 70 $\mu\text{g Cu/L.}$ (average 40) were analyzed in duplicate. The ratio of the higher result to the lower in each set averaged 1.02

and ranged from 1.00 to 1.09. The coefficient of variation of the method calculated from the replicates averaged 1.6% and ranged from 0% to 6%.

The results of all measurements of the background level of copper in undosed Shayler Run water are given in Table 2. These values were not corrected for the recovery percentages. Set 1 of the measurements was performed on grab samples collected almost weekly at station 6 until July 30, 1968, and at station 1 afterwards. Set 2 of the measurements was performed in triplicate on grab samples in conjunction with some of the recoveries.

The other sets of measurements were performed on composite samples formed by combining equal volumes of water taken on 7 consecutive days from the control test containers from chronic tests conducted at streamside. Sets 4A and 4B were taken from two different chronic tests conducted simultaneously. For the 20 weeks for which samples were collected for both sets, the ratio of 4A to 4B averaged 0.92, with a standard deviation of 0.23, and ranged from 0.65 to 1.52.

Similarly, sets 5A and 5B were taken from two different simultaneous chronic tests. For the 16 weeks for which samples were collected for both sets, the ratio of 5A to 5B averaged 1.06, with a standard deviation of 0.31, and ranged from 0.49 to 1.78.

Samples for sets 6A and 6B were taken from one chronic test, whereas samples for set 6C were taken from a simultaneous test. Although set 6A was analyzed by the extraction procedure, sets 6B and 6C were analyzed by direct aspiration. For the duplicate analyses performed on 22 samples, the ratio of 6A to 6B averaged 1.46, with a standard deviation of 0.49, and ranged from 0.76 to 2.83. For the 20 weeks for which samples were analyzed for both sets, the ratio of 6B to 6C averaged 1.04, with a standard deviation of 0.36, and ranged from 0.50 to 1.80.

To determine the concentration of copper in the exposure area of Shayler Run when the stream was being dosed as desired, water samples were collected in 125-ml polyethylene water-tight bottles that had been rinsed with 10% concentrated nitric acid. Normally, samples were collected in Monday, Wednesday, and Friday if the stream was being dosed. Whenever dosing was interrupted for any reason, samples were not collected for 24 hr after dosing was begun again. Three sampling schedules were used during the study. From February 16, 1970, to September 15, 1970, about 20% of the sample collections consisted of duplicate samples at stations 3, 4, 5, and 6, and the rest of the collections consisted of single samples at each of these stations. From September 15, 1970, to June 1, 1971, collections consisted of duplicate samples at stations 3 and 6 and single samples at stations 4 and 5. From June 1, 1971, to October 31, 1972, collections consisted of duplicate samples at stations 2, 3, and 6 and single samples at stations 4 and 5. Samples were taken near the middle of the stream, below the surface and upstream of the collector. Samples were transported to the laboratory, acidified with 4 drops of concentrated nitric acid, and shaken. They were then analyzed for copper by direct aspiration atomic absorption spectrophotometry and were compared against standards containing similar levels of copper prepared in 0.15% concentrated nitric acid in distilled water.

Between May 10, 1970, and January 11, 1973, 57 measurements of the accuracy of the direct aspiration method were made at each of two levels by adding equal amounts of copper to both acidified Shayler Run water and acidified distilled water. For 100 $\mu\text{g Cu/L}$, the increase in instrumental response for the Shayler

TABLE 2. BACKGROUND COPPER CONCENTRATIONS IN SHAYLER RUN WATER, 1968-73
($\mu\text{g}/\text{l.}$)

Set	Beginning dates	Ending dates	Number of samples	Mean	Standard deviation	Range mg/l.
1	5-29-68	10-13-69	59	5.0	3.2	1-18
2	3-30-71	12-3-71	7	4.4	2.9	3-6
3	1-21-70	9-9-70	32	6.9	2.3	4-14
4A	9-30-70	4-21-71	26	6.3	1.9	3-10
4B	10-14-70	3-3-71	20	5.5	1.5	3-9
5A	4-28-71	8-25-71	16	6.4	3.1	4-15
5B	4-28-71	8-25-71	16	6.4	3.1	4-17
6A	1-12-72	10-4-72	22	6.7	3.6	4-21
6B	1-12-72	1-18-73	34	9.2	3.8	5-24
6C	1-26-72	8-23-72	20	8.3	2.5	5-14

Run water, compared to that for the distilled water, averaged 96.4% with a standard deviation of 3.1%, after one value of 115% was discarded as an outlier. For 400 $\mu\text{g Cu/L}$, the increase for the Shayler Run water, compared to that for the distilled water, averaged 96.6% with a standard deviation of 2.0%.

For 680 sets of duplicate samples of dosed Shayler Run water containing between 30 and 263 $\mu\text{g Cu/L}$, obtained from stations 2, 3, 4, 5, and 6 between February 16, 1970, and October 31, 1972, the ratio of the higher result to the lower averaged 1.04 and ranged from 1.00 to 1.56 after one value of 2.27 was discarded as an outlier. The coefficient of variation calculated from these duplicates averaged 4.8% and ranged from 0% to 30% after one value of 55% was discarded as an outlier.

The monthly averages of the measured copper concentrations in dosed Shayler Run water are given in Table 3. These values were not corrected for the results of the method of known additions. The results of 30 of the more than 2,100 analyses were discarded because they were not consistent with the rest of the values obtained for the same day. Only four values were above 195 $\mu\text{g/L}$ (258 and 263 at station 2 on July 28, 1971, and 234 and 239 at station 2 on June 21, 1972), and these were discarded. No values were below 30 $\mu\text{g/L}$. The monthly standard deviations averaged 11.1 and ranged from 1 to 28. They tended to be higher in the summer, lower at the downstream stations, and higher near the beginning of the project.

TABLE 3. MONTHLY AVERAGE COPPER CONCENTRATIONS IN EXPOSURE SECTION OF SHAYLER RUN ($\mu\text{g/l.}$)

Month	2	3	4	5	6	6/3 ^a	Number of days sampled
1970							
Feb.	-	111.0	106.9	101.2	99.5	89.3	15
March	-	103.8	97.0	90.2	87.4	86.3	10
April	-	109.5	102.6	88.9	85.7	79.3	11
May	-	122.5	101.8	79.3	75.0	61.8	15
June	-	106.8	84.8	53.3	48.8	47.6	12
July	-	104.1	81.4	53.2	51.2	48.8	12
Aug.	-	113.5	83.9	57.3	52.1	44.5	10
Sept.	-	131.5	99.2	67.2	58.1	44.4	13
Oct.	-	132.5	119.5	96.6	83.7	64.0	12
Nov.	-	114.7	111.0	98.8	94.4	82.3	13
Dec.	-	116.8	108.8	102.4	96.3	83.4	10
1971							
Jan.	-	117.4	115.4	111.3	111.2	96.3	10
Feb.	-	134.7	128.2	119.1	111.4	-	2
March	-	108.5	103.1	95.2	92.5	85.0	6
April	-	109.0	98.6	86.1	81.3	74.9	11
May	-	105.9	93.3	79.3	76.3	73.3	10
June	135.9	123.6	99.6	70.8	69.1	53.3	13
July	138.3	130.5	100.2	73.3	64.9	51.0	10
Aug.	140.6	122.3	96.7	65.1	60.5	48.5	12
Sept.	117.5	108.6	102.7	86.5	73.5	73.9	7
Oct.	135.8	122.6	108.0	90.9	86.6	70.8	12
Nov.	120.1	112.6	103.3	90.2	86.2	76.0	13
Dec.	98.7	96.4	91.0	83.6	82.8	86.0	8
1972							
Jan.	102.6	99.3	94.1	89.0	88.0	85.1	7
Feb.	114.2	111.4	106.6	105.1	102.1	-	4
March	96.2	97.3	88.6	87.8	83.8	86.3	8
April	108.6	102.2	94.3	86.6	81.4	-	3
May	113.5	108.2	101.5	88.1	81.4	73.1	9
June	120.6	115.2	99.9	81.7	75.1	64.6	12
July	151.3	137.6	104.0	75.8	64.5	49.1	13
Aug.	154.7	130.7	97.8	63.3	56.1	42.9	7
Sept.	168.6	141.5	110.9	72.5	62.2	44.1	7
Oct.	142.5	131.0	117.6	98.2	94.6	72.2	8

^aPercent copper in the stream at station 6 compared to station 3, the point of thorough toxicant mixing.

PART B -- FIELD STUDIES

SECTION X

EFFECTS OF COPPER ON STREAM FISH

INTRODUCTION

Many collections and observations were made to evaluate the effects of the addition of copper on population density, reproduction, growth, survival, and avoidance or other behavioral reactions of fish. The purposes of the biannual fish collections were to ascertain base population levels in control and exposure areas before and after introduction of copper; to determine in the spring whether mature fish were present in these areas before their spawning period; and to determine in the fall the degree of spawning success and survival of young.

Since fish weirs were installed to isolate the exposure area, changes in fish activity resulting from the addition of copper would be evident. If fish avoided copper in the stream or their activity increased or both, greater numbers would be expected on the exposure weir. A species comparison of fish captured could then be made with those captured on the upstream control weir. The purpose of spawning observations was to obtain data relating to the effects of copper, if any, on fish spawning success and spawning behavior.

Sections in this part of the report will deal with the collections and observations used to evaluate the effects of copper on the fish populations. In Part D of this report these field results will be related to laboratory results obtained before and during the study.

METHODS

Biannual Fish Collections

Eight biannual (spring and fall) collections were made, starting in the spring of 1968 and ending in the fall of 1971. Four collections were made before the introduction of copper, and four collections were made during the exposure period. The collections were made in the same pool and riffle areas each time (Figure 3).

Collections were made with an electrofishing apparatus consisting of an electric seine, 7.6 m long, similar to the one described by Funk (1957). A variable voltage pulsator (Coffelt Electronic Co., Model No. III-C) was used, and the power supply was a Model 9A 115-1A Homelite generator with a continuous output of 3,000 watts. The variable voltage pulsator supplied both alternating current (AC) and direct current (DC), but AC was more successful in this stream and was used for all collections. The AC voltage could be varied between 0 and 280 with this unit. Since all fish captured were removed for further analysis, little concern was given to killing or injuring the specimens with an electric charge. The maximum electric output of the system was always used.

Block seines made from 4.76-mm stretch mesh were placed at the upstream and downstream limits of each section of stream being fished to prevent escapement. Collections were made with a crew of six men only during periods of low flow and when the water was clear. Two passes were made over each area, and an attempt was made to capture all fish seen. During the initial collection period a third pass of an area produced less than 2% of the total catch from the two previous passes and was subsequently abandoned.

All collected specimens were preserved with acetic acid formalin and alcohol (AFA), and specimens 60 mm and longer were injected with AFA so that stomach contents would be well preserved for future analysis. Fish were identified to species, enumerated, and measured. For the eight most abundant species length-frequency graphs were prepared as an aid in determining age-group 0. Age-group 0 was defined as those individuals that were less than 1 year old in a fall collection and between 7 and 13 months old in a spring collection. All fish that were older at the designated time were considered adults.

Weir-Screen Collections

Daily collections were made from the weir screens, beginning 2 days before the introduction of copper sulfate to the stream. During the period of exposure February 16, 1970, through October 31, 1972, few fish other than fry were taken on the screens between June and February. During May many predators and scavengers consumed fish captured on the screens. Data presented are from the dates within the period February 15 to May 28. The initial sampling dates were dependent upon flooding and icing conditions and the ending dates upon activities of predators and scavengers.

Fry Collections (Traps and Weirs)

Fry samples were collected during the exposure period from fish weirs and fry traps to obtain information relating to reproduction and young-of-the-year growth rates in control and exposure areas.

The fry were captured from both areas for identification. This sampling was only qualitative. Fry captured on the fish weirs could not be compared because the collections of fish from the weirs did not include all species of fry observed in the study stream. Many of the specimens were partially decomposed, and scavengers or predators removed fry from the weirs.

In 1971 fish-fry traps were designed and built to obtain both quantitative and qualitative samples and specimens in better condition, but too few fry were captured in the exposure area for quantitative estimates. These traps were constructed of fine mesh brass window screen and aluminum framing (Figure 10). The trap was placed at the stream's edge, where the waters were shallow and of low velocity and the bottom substrate was composed of fine sand and some silt. A 2-m lead touched the shore above the water's edge, and a short lead (1 m) was placed at right angles to the shore. An opening of approximately 15 cm remained between the short lead and the shore. At the termination of a 4-hr sampling period, a piece of sheet metal was placed across this opening and anchored in the stream bottom. The fry were then removed from the trap with a fine-mesh dip net and preserved. In the laboratory they were identified to species, measured for length, and counted.

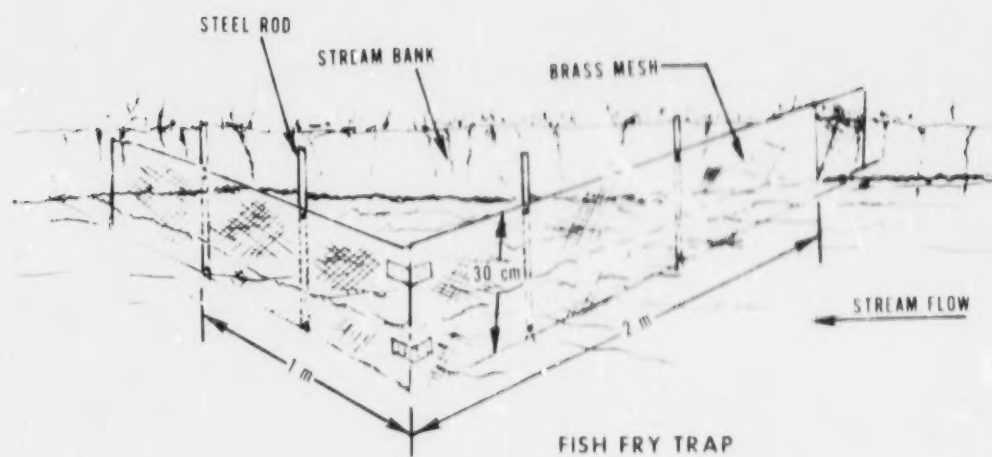


Figure 10. Schematic of fish-fry trap.

Three similar locations in both the control and exposure area were selected for trap placement (Figure 3). All traps were fished for 4 hr over the same general period. Twenty-two collections were taken starting on May 19, 1971, and ending on August 30, 1971.

Fish-Spawning Observations

Fish spawning was observed for the years 1968 through 1972. Considerable time was spent during the pre-exposure period observing spawning activity of various species, but the number of spawns was not recorded. For every spawning activity observed and recorded, a confirmation of the spawn was made by examining the eggs for fertility and age. For species that spawn in groups, such as stoneroller, creek chub, striped shiner, and white sucker, each group was recorded as one spawning. The range in number of individuals per group for the above four species was 20-30, 5-10, 20-25, and 10-15, respectively. For bluntnose minnows, which spawn on the underside of rocks, one group of eggs was recorded as one spawning.

Spawning observations were usually made every other day, but varied with stream conditions. During the exposure period observations were normally made for equal lengths of time (1 to 1-1/2 hr) in both control and exposure areas when a species was known to be spawning. More time and effort were spent on species that have secretive spawning habits, such as the bluntnose minnow, and emphasis was placed on finding first spawning of each species. On numerous occasions spawning activity was prevented by an abrupt decrease in temperature.

OBSERVATIONS AND RESULTS

Avoidance

On February 22, 1970, approximately 400 fish were seen concentrated at a point in the exposure area where a small spring-fed stream enters. Most were striped shiners, stonerollers, and bluntnose minnows, but a few sunfish and darters were also present. The copper concentration in the area where the fish were seen was 77 $\mu\text{g}/\text{l}$., whereas the copper concentration at midstream at this location was 121 $\mu\text{g}/\text{l}$.. The temperature of the spring water was 2° C, and the stream was 1° C at the mouth of a tributary in the control stretch. No fish of any species were observed and the temperature of the tributary was 3° C, indicating that the congregation of fish was not caused by a temperature preference. Few fish were seen in the control area other than darters, which raced away when stream rocks were disturbed, and schools of striped shiners at the heads of deeper pools. On the other hand, numerous fish were observed in the exposure area located at the very edge of the stream or in small shallow backwater pools. The copper concentration in one of these pools measured 3 $\mu\text{g}/\text{l}$., but in midstream at this location it was 125 $\mu\text{g}/\text{l}$..

A decision was made to introduce control water 130 m downstream from the point of copper introduction to verify the apparent avoidance. No fish had been observed at this site on February 22. On February 23, 3 mature creek chubs, 10 mature stonerollers, 8 rainbow darters, 20 bluntnose minnows, and 3 mature striped shiners were observed at the location of this discharge. At the spring outfall in the exposure stretch for that date, estimates were 300-400 mature striped shiners, 40-50 stonerollers, 200-300 bluntnose minnows, and a few sunfish. By the first of May and thereafter, no concentrations of fish were observed at the spring outfall or along the stream edge or in backwaters, and those fish that were

observed in the exposure area were in what would be considered their normal habitat, suggesting that either the animals adapted to the copper in the stream or they were under a greater physiological stress at the low water temperatures and short photoperiod.

For the first week of dosing, no fish were observed dead or in distress; however, on February 23, 7 days after the start of exposure, and for approximately 1 week thereafter, fish were observed dead or in distress in the exposure area, but not in the control area. The following fish were observed for that period:

	Dead	Distressed
Stoneroller	20	13
Hog sucker	9	2
Johnny darter	4	1
Bluntnose minnow	2	7
White sucker	1	1
Carp	-	1
Green sunfish	1	-

Since no fish were observed dead or in distress in the control area, copper apparently caused the above effects, especially to the more sensitive stonerollers, hog suckers, johnny darters, and bluntnose minnows.

In other tests stonerollers were shown to be the most sensitive and died in the greatest numbers. The actual number observed was small when compared to the stream population. Since numerous crayfish in the exposure area consumed dead fish most readily, the number of fish killed by copper was probably greater than observed.

Biannual Fish Collections

Thirty-four species of fish were collected during the study period. The totals for the control and exposure areas for these eight collections are presented in Table 4. The first eight species listed lend themselves to more detailed analysis since adequate populations of these were present in both control and exposure areas during the pre-exposure period. The ninth species listed, longear sunfish, was not common in the control area, but was common in the exposure area in adequate numbers for evaluation. The last 25 species were considered rare or not common to the stream. Some of them may have escaped from upstream ponds that drained to Shayler Run.

Table 5 presents the number of age-group 0 and mature fish by species for control and exposure areas from the biannual collections. The mature portion of the fish population was more stable than the age-group 0 portion during the pre-exposure period. This would be expected in a natural population because of the various stresses on spawning success and the vulnerability of eggs and young to flooding and predation. Figures 11A through 18A present graphically the numbers of adults of the eight most numerous species collected from control and exposure areas.

Except in the 1970 fall collection, bluntnose minnow adults (Figure 11A) show a general decline in the exposure area during the exposure period when compared with the pre-exposure period. Further, the population of adult bluntnose

TABLE 4. TOTAL NUMBER OF INDIVIDUALS COLLECTED FROM SHAYLER RUN IN THE BIENNIAL FISH COLLECTIONS, 1968-71

Species ^a	Location	April		Sept.		March		Sept.		Feb.		May		Oct.	
		1968	1969	1968	1969	1969	1969	1969	1970	1970	1971	1971	1971	1971	
1. Bluntnose minnow <u>Pimephales notatus</u> (Rafinesque)	Control Exposure	335 278	2,057 465	922 765	1,643 429	648 152	818 808	315 51	332 237						
2. Striped shiner <u>Notropis chryscephalus</u> (Rafinesque)	C ^b E ^c	356 346	2,965 596	1,323 1,121	436 493	190 217	360 268	72 113	236 73						
3. Creek chub <u>Semotilus atromaculatus</u> (Mitchill)	C E	136 118	287 834	213 104	262 133	289 42	423 157	178 133	570 116						
4. Stoneroller <u>Campestenoma anomalum</u> (Rafinesque)	C E	101 139	279 551	1,026 184	531 496	112 51	1,322 182	312 76	402 34						
5. Rainbow darter <u>Etheostoma caeruleum</u> Storer	C E	96 162	57 187	33 153	14 60	8 11	46 28	0 22	5 3						
6. Fantail darter <u>Etheostoma flabellare</u> Rafinesque	C E	166 193	66 131	61 203	19 75	30 19	136 281	45 121	52 65						
7. Orangefthroat darter <u>Etheostoma spectabile</u> (Agassiz)	C E	98 81	183 72	129 231	137 50	13 45	148 128	58 138	159 247						
8. Green sunfish <u>Lepomis cyanellus</u> Rafinesque	C E	78 133	78 156	24 74	31 39	36 70	128 46	17 52	42 70						
9. Longear sunfish <u>Lepomis megalotis</u> (Rafinesque)	C E	47 83	8 248	0 58	0 125	1 78	0 79	0 36	5 22						
10. Johnny darter <u>Etheostoma nigrum</u> Rafinesque	C E	19 20	23 3	23 8	18 16	36 31	76 62	46 29	60 20						
11. Blacknose dace <u>Rhinichthys atratulus</u> (Hermann)	C E	2 1	63 11	154 20	49 18	22 1	108 12	72 14	90 10						

TABLE 4 (continued). TOTAL NUMBER OF INDIVIDUALS COLLECTED FROM SHAYLER RUN
IN THE BIENNIAL FISH COLLECTIONS, 1968-71

Species	Location	April '68	Sept. '68	March '69	Sept. '69	Feb. 16-1970 copper first introduced to exposure area	May '70	Oct. '70	April '71	Oct. '71
12. White sucker <u>Catostomus commersoni</u> (Lacépède)	C E	68 84	64 53	10 35	0 3	0 3	30 14	17 8	12 16	90 129
13. Rosefin shiner <u>Notropis ardens</u> (Cope)	C E	6 17	4 177	0 11	0 199	0 0	0 4	0 26	0 1	0 12
14. Northern hog sucker <u>Hypentelium nigricans</u> (Lesueur)	C E	11 17	5 9	0 6	0 23	0 0	2 0	4 5	6 0	4 0
15. Bluegill <u>Lepomis macrochirus</u> Rafinesque	C E	17 13	38 226	1 0	19 112	0 0	45 6	287 15	0 0	0 0
16. Golden redhorse <u>Moxostoma erythrum</u> (Rafinesque)	C E	26 19	0 5	0 0	0 0	0 0	0 0	0 0	0 0	0 0
17. Black bullhead <u>Ictalurus melas</u> (Rafinesque)	C E	22 20	0 18	0 1	0 0	0 0	0 1	0 0	1 0	0 0
18. Gizzard shad <u>Porosoma cepedianum</u> (Lesueur)	C E	0 0	0 3	0 0	0 0	0 0	0 0	0 0	0 0	0 0
19. Spotted sucker <u>Minytrema melanops</u> (Rafinesque)	C E	0 0	0 2	0 0	0 0	0 0	0 0	0 0	0 0	0 0
20. Carp <u>Cyprinus carpio</u> Linnaeus	C E	15 0	0 0	0 1	0 0	0 0	0 0	0 0	0 0	0 0
21. Golden shiner <u>Notemigonus crysoleucas</u> (Mitchill)	C E	1 1	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
22. Spottfin shiner <u>Notropis spilopterus</u> (Cope)	C E	1 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
23. Silverjaw minnow <u>Ercymba buccata</u> Cope	C E	3 2	0 6	0 14	0 0	0 0	0 0	0 0	0 0	0 0

TABLE 4 (continued). TOTAL NUMBER OF INDIVIDUALS COLLECTED FROM SHAYLER RUN
IN THE BIENNIAL FISH COLLECTIONS, 1968-71

Species ^a	Location	April '68	Sept. '68	March '69	Sept. '69	Feb. 16-1970 copper first introduced to exposure area	May '70	Oct. '70	April '71	Oct. '71
24. Fathead minnow <u>Pimephales promelas</u> Rafinesque	C E	0 1	0 0	0 0	0 0	0 0	11 0	1 0	0 0	0 0
25. Yellow bullhead <u>Ictalurus natalis</u> (Lesueur)	C E	0 6	1 25	0 8	0 0	0 0	1 1	0 1	0 3	0 0
26. Brown bullhead <u>Ictalurus nebulosus</u> (Lesueur)	C E	2 1	1 11	0 1	0 0	0 0	0 0	0 0	0 0	0 0
27. White crappie <u>Pomoxis annularis</u> Rafinesque	C E	0 0	3 0	0 0	0 0	0 0	19 0	8 3	0 0	0 0
28. Rock bass <u>Ambloplites rupestris</u> (Rafinesque)	C E	0 3	1 6	0 0	0 0	0 0	0 0	0 0	0 0	0 0
29. Smallmouth bass <u>Micropterus dolomieu</u> Lacépède	C E	1 4	0 61	0 24	0 0	0 0	0 0	0 0	0 0	0 0
30. Spotted bass <u>Micropterus punctulatus</u> (Rafinesque)	C E	1 3	14 18	0 0	0 0	0 0	0 0	0 0	0 0	0 0
31. Largemouth bass <u>Micropterus salmoides</u> (Lacépède)	C E	0 2	0 4	0 0	0 0	0 0	3 2	0 1	0 0	0 0
32. Greenside darter <u>Etheostoma blennioides</u> Rafinesque	C E	3 2	0 0	0 0	0 0	0 0	0 0	1 7	0 2	0 3
33. Emerald Shiner <u>Notropis antherinoides</u> Rafinesque	C E	0 0	0 0	0 0	0 0	0 0	0 1	0 0	0 0	0 0
34. Northern redbelly dace <u>Chrosomus eos</u> Cope	C E	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 1

^aBailey, et al. (1970).

^bControl.

^cExposure.

TABLE 5. AGE-GROUP 0 AND MATURE (IN PARENTHESIS) FISH COLLECTED FROM SHAYLER RUN IN BIENNIAL FISH COLLECTIONS, 1968-71

Species	Location	Pre-exposure period			Feb. 16-1970 copper first to exposure area	Exposure period		
		April '68	Sept. '68	March '69		May '70	Oct. '70	Oct. '71
Bluntnose minnow	Control Exposure	153(182) 173(125)	1,902(155) 320(145)	845(77) 570(195)		386(262) 112(40)	544(274) 575(263)	221(94) 42(9)
Striped shiner	C ^a E ^b	44(312) 42(304)	2,489(76) 411(185)	1,298(27) 774(347)	152(284) 5(488)	49(141) 40(177)	21(339) 104(164)	30(42) 5(108)
Creek chub	C E	65(71) 46(72)	170(117) 268(166)	161(52) 29(75)	193(69) 33(96)	128(160) 20(22)	301(122) 104(53)	127(51) 92(41)
Stoneroller	C E	35(66) 47(92)	688(91) 202(349)	940(86) 137(47)	375(156) 138(358)	50(62) 23(28)	981(341) 81(101)	207(105) 61(15)
Rainbow darter	C E	62(34) 104(58)	33(24) 30(157)	33(20) 78(75)	7(7) 5(55)	2(6) 7(4)	12(34) 6(22)	1(8) 12(10)
Fantail darter	C E	57(109) 72(121)	17(49) 41(90)	24(37) 83(122)	3(16) 9(66)	16(14) 11(8)	73(63) 233(48)	14(31) 83(38)
Orangethroat darter	C E	69(32) 72(20)	170(13) 41(31)	107(22) 149(82)	118(19) 24(26)	10(3) 36(9)	100(48) 103(25)	40(18) 91(47)
Green sunfish	C E	26(52) 43(73)	20(58) 17(139)	19(5) 34(40)	2(29) 14(25)	24(12) 45(25)	5(123) 5(41)	0(17) 9(43)

^aControl.
^bExposure.

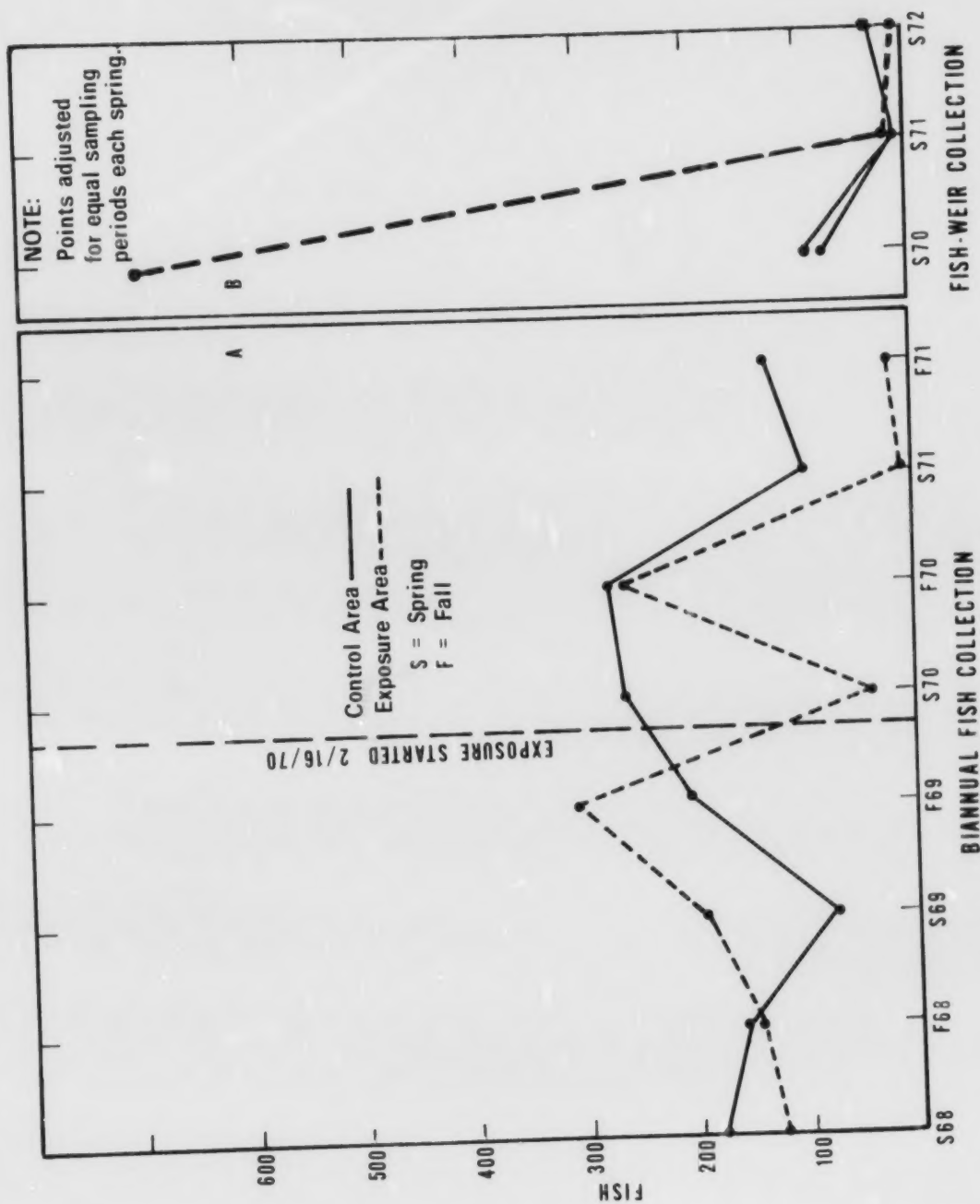


Figure 11. Numbers of mature bluntnose minnows from (A) biannual fish collections and (B) weir-screen collections.

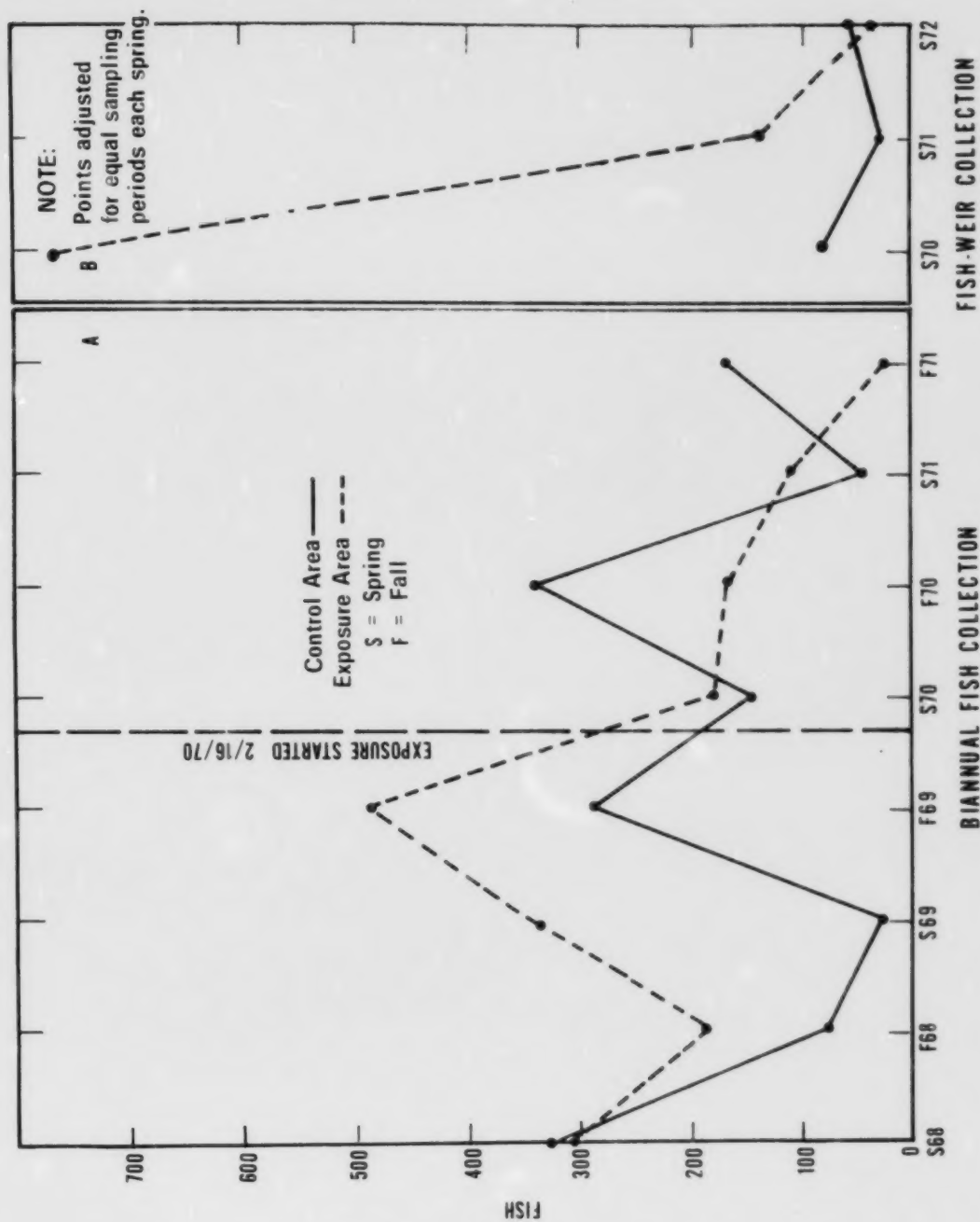


Figure 12. Numbers of mature striped shiners from (A) biannual fish collections and (B) weir-screen collections.

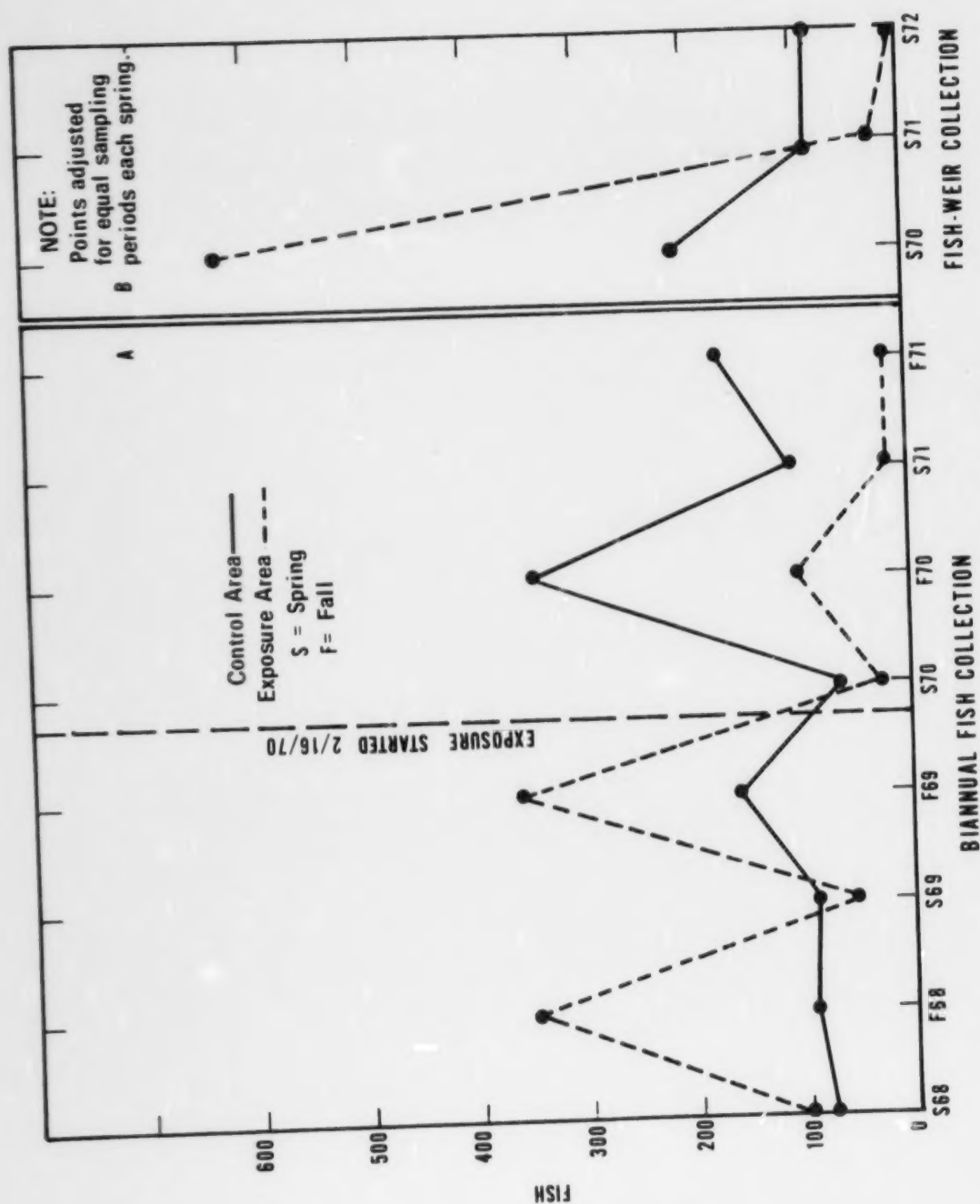


Figure 13. Numbers of mature stonerollers from (A) biannual fish collections and (B) weir-screen collections.

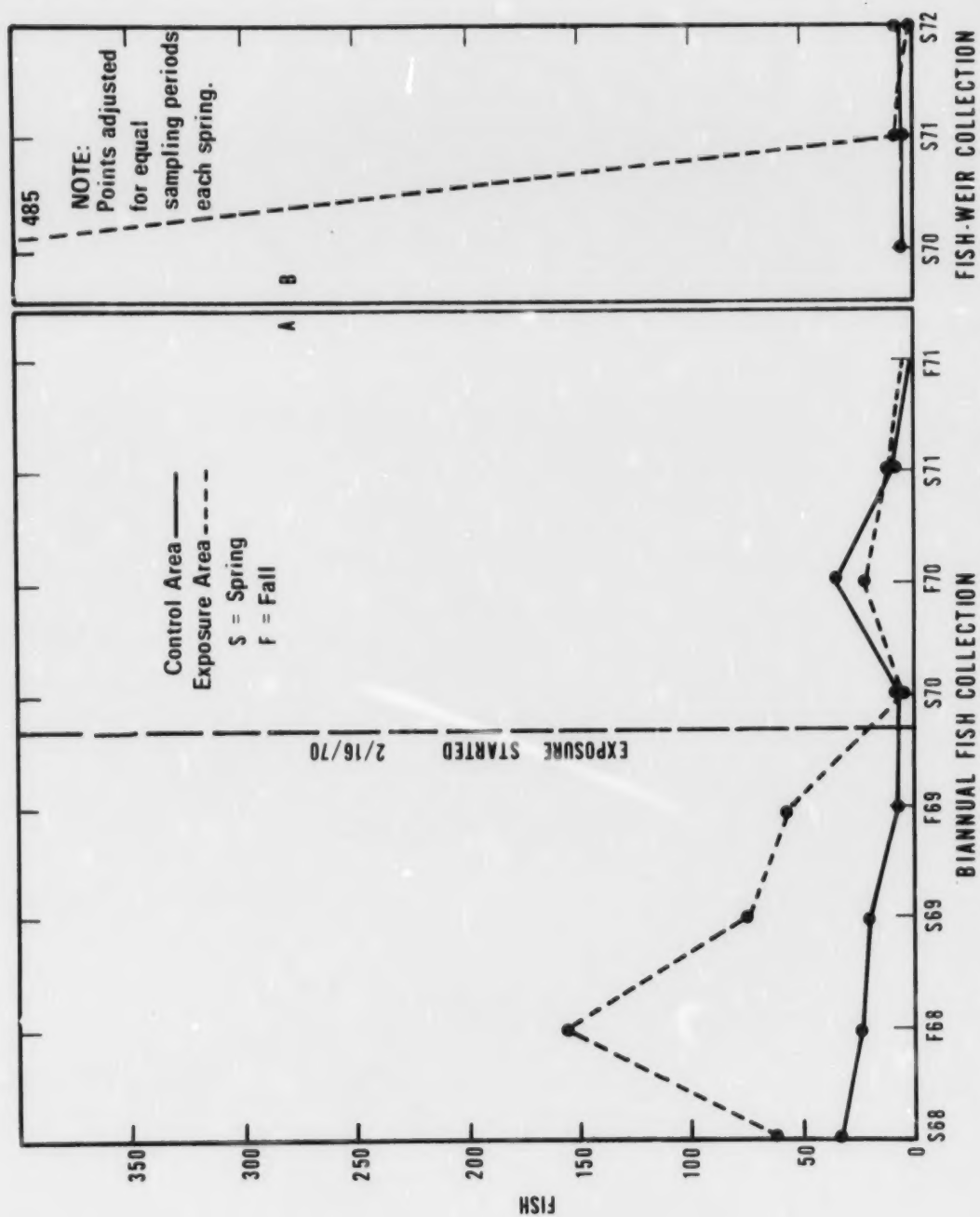


Figure 14 Numbers of mature rainbow darters from (A) biannual fish collections and (B) weir-screen collections.

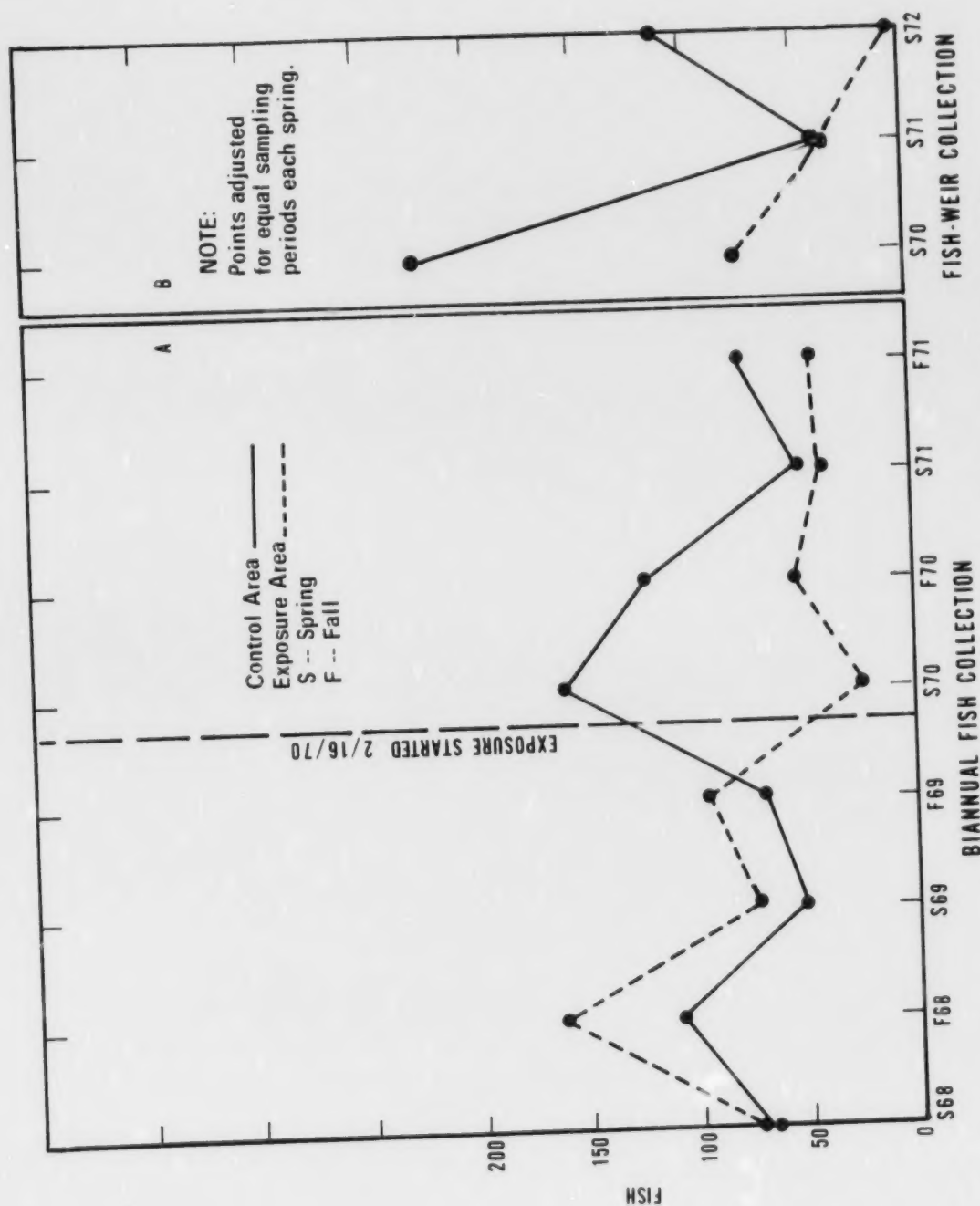


Figure 15. Numbers of mature creek chubs from (A) biannual fish collections and (B) weir-screen collections.

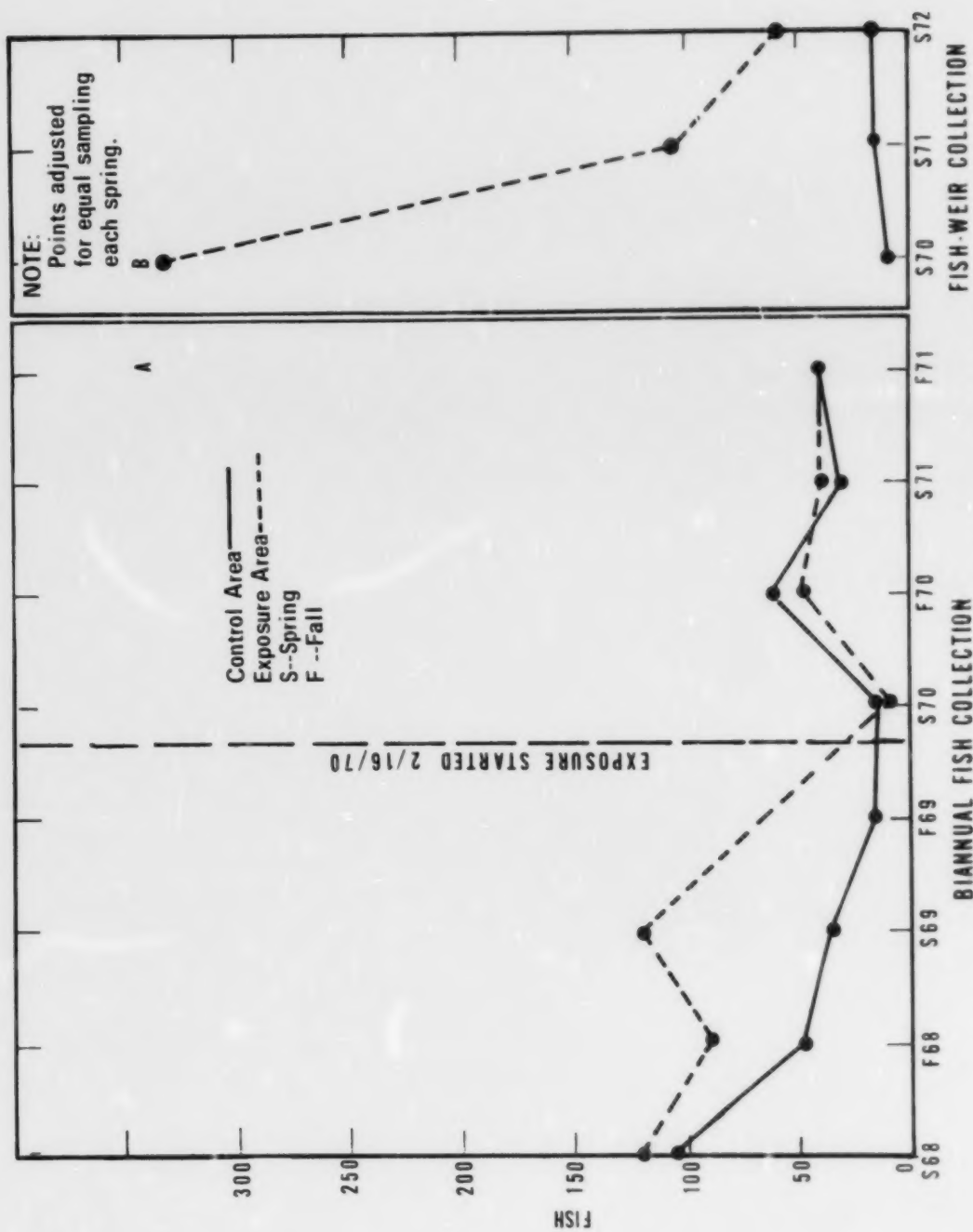


Figure 16. Numbers of mature fantail darters from (A) biannual fish collections and (B) weir-screen collections.

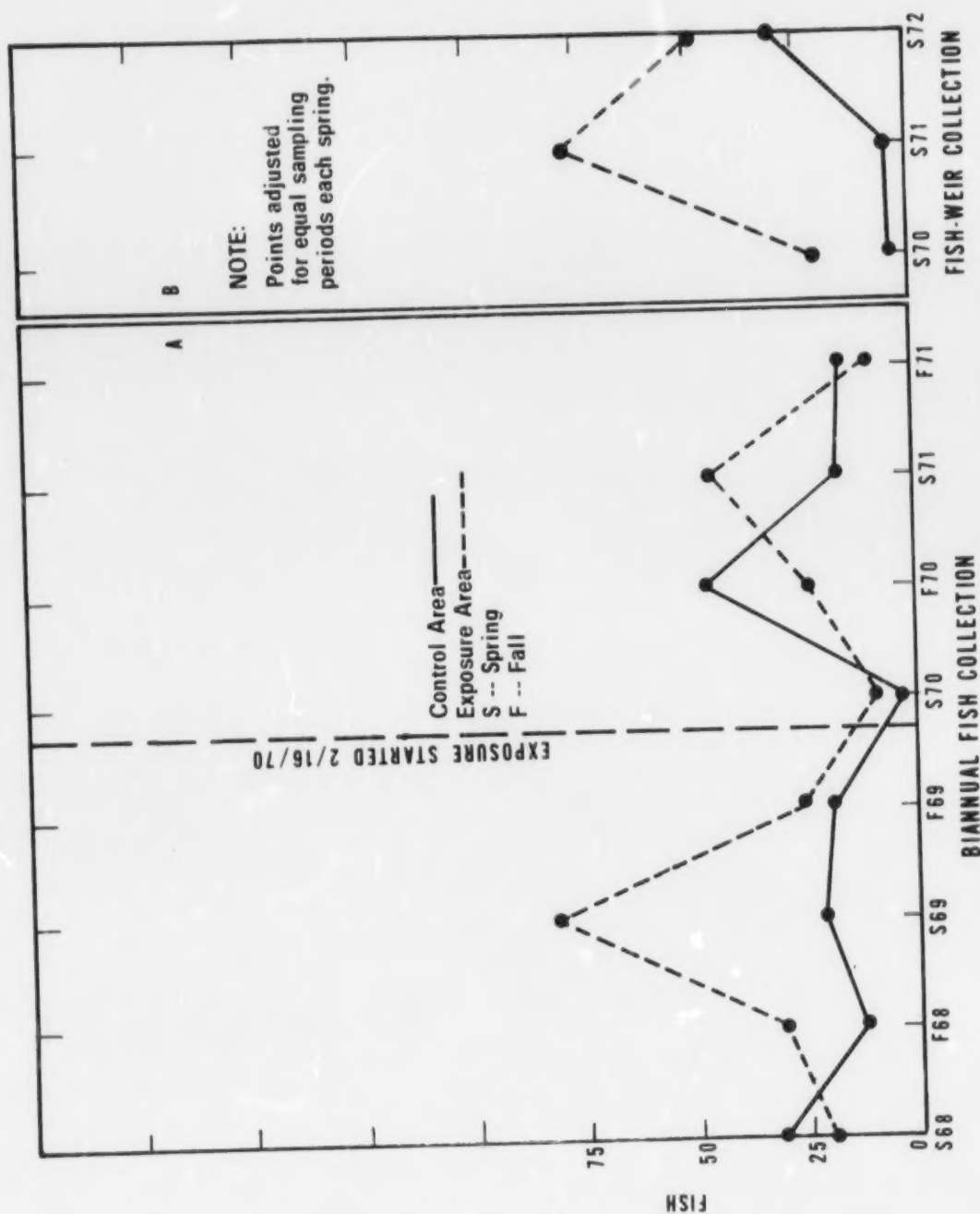


Figure 17. Numbers of mature orangethroat darters from (A) biannual fish collections and (B) weir-screen collections.

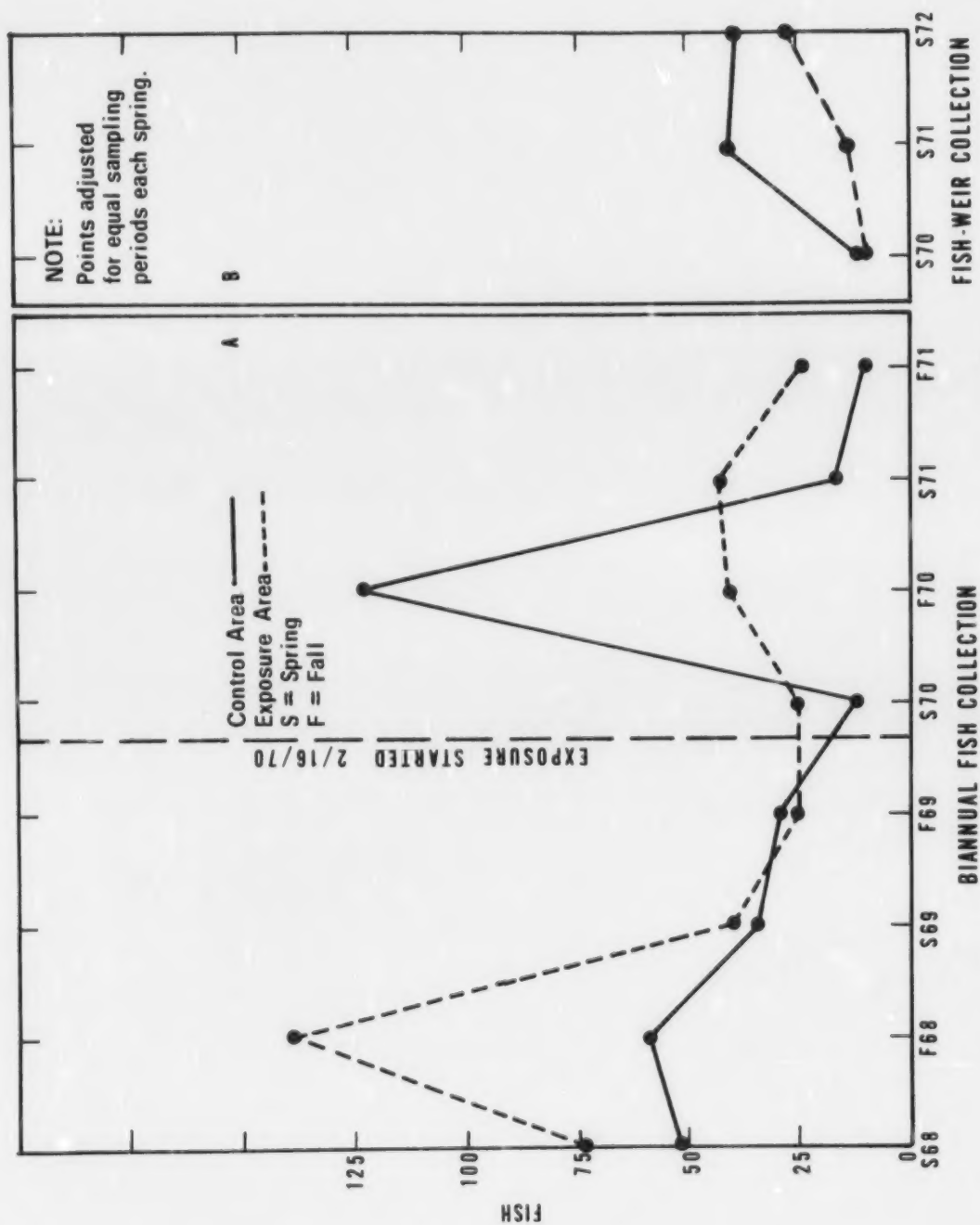


Figure 18. Numbers of mature green sunfish from (A) biennial fish collections and (B) weir-screen collections.

minnows in the spring and fall collections of 1971 was considerably lower than that of the lowest collection during the pre-exposure period.

The numbers of adult striped shiners (Figure 12A), stonerollers (Figure 13A), and rainbow darters (Figure 14A) represented in the exposure collections for the dosing period show a definite decline without any appreciable recovery when compared with numbers for the predosing period.

Creek chub and fantail darter adults (Figures 15A and 16A) in the exposure area display a general decline in numbers in the collections during the exposure period when compared with those for the pre-exposure period. Adult orangethroat darter and green sunfish populations (Figures 17A and 18A) did not appear to be directly affected by copper during the exposure period.

Weir-Screen Collections

Information is presented in Table 6 that pertains to the addition of copper to the stream, the flooding of the screens, and the number of fish collections from the weir screens during the exposure period between February 15 and May 28, 1970-72. Data for adults of the eight most abundant fish species are graphically expressed in Figures 11B through 18B.

During the first spring of copper introduction greater numbers of adult bluntnose minnows, striped shiners, stonerollers, rainbow darters, and fantail darters were captured on the exposure screens than on the control screens (Table 7). In the following two springs, however, when the copper was still being added, the numbers of adults captured from control and exposure areas were similar. This may have been due to acclimation to copper or to fewer fish in the exposure area. Young-of-the-year bluntnose minnows and rainbow darters also contributed large numbers of individuals to collections from the exposure screens during the first spring of dosing.

Numbers of adult green sunfish, longear sunfish, and orangethroat darters on both screens were low. However, the numbers of young-of-the-year orangethroat darters on the exposure screens substantially increased during the springs of 1971 and 1972 compared to 1970. In fact, the number of young-of-the-year captured by the exposure screen during the spring of 1972 on 21 collection days was approximately seven times greater than the number captured in 1970 on 53 collection days. The downstream movement of young-of-the-year orangethroat darters from both the control and exposure areas increased during the three springs of exposure (Table 8). The number of individuals captured on the exposure screens progressively increased each year during the period of exposure, even on those days when copper was not added to the stream. Thus this migration of young-of-the-year orangethroat darters was not directly related to copper exposure. For example, in the spring of 1972, 293 specimens were captured on the exposure weir screen in 21 days of copper exposure. In contrast, 221 specimens were captured from the same weir in 15 days when no copper was being added. The cause of this movement is not known; it may be a normal downstream migration. Results of daily weir-screen collection for 1970 (Figure 19) present a more detailed account of fish movement and how the various species responded to copper.

The bluntnose minnow and, to a limited degree, the stoneroller gave an initial response to copper within the first 48 hr of exposure by moving out of the exposure area onto the screens. These two species, plus the striped shiner,

TABLE 6. DATA RELEVANT TO THE FISH COLLECTIONS
ON THE WEIR SCREENS, SHAYLER RUN, 1970-72

Year	1970	1971	1972
Dates of collections	2/15-5/12	2/25-5/28	3/11-5/3
No. of days copper was added	58	69	23
No. of times weirs flooded	5	6	7
Total hours of flooding	140	114	163
No. of fish collections when adding copper	53	56	21
No. of fish collections when not adding copper	8	16	15

TABLE 7. NUMBER OF AGE-GROUP 0 AND ADULT FISH COLLECTED FROM SHAYLER RUN ON WEIR SCREENS^a

Species	Location	Spring 1970(53) ^b		Spring 1971(56) ^b		Spring 1972(21) ^b		Spring 1970(4) ^b		Spring 1971(16) ^b		Spring 1972(15) ^b	
		Age-group 0	Adult	Age-group 0	Adult	Age-group 0	Adult	Age-group 0	Adult	Age-group 0	Adult	Age-group 0	Adult
Bluntnose minnow	Control	56	74	34	9	10	14	2	7	8	5	3	2
	Exposure	460	579	20	15	32	8	3	7	2	0	1	1
Striped Shiner	C ^c	5	69	2	19	0	12	0	6	0	9	0	15
	E ^d	34	674	0	138	5	17	0	5	0	14	0	2
Creek chub	C	17	178	1	30	0	35	2	12	1	10	1	25
	E	6	60	1	37	0	3	0	1	1	0	0	1
Stoneroller	C	32	152	89	59	11	25	0	29	77	28	27	22
	E	67	561	91	35	14	3	0	6	25	11	1	4
Rainbow darter	C	8	3	4	3	1	5	1	1	0	0	3	5
	E	260	415	3	9	8	0	0	3	8	5	2	2
Fantail darter	C	1	9	7	15	3	3	0	2	12	10	14	12
	E	86	315	90	84	11	6	0	3	46	26	29	28
Orangethroat darter	C	11	4	10	2	31	7	0	1	11	3	21	8
	E	38	13	89	62	293	21	4	3	56	37	221	3
Green sunfish	C	12	12	0	26	1	2	1	0	0	17	3	16
	E	7	10	0	10	0	9	0	0	0	4	1	5
Longear sunfish	C	4	3	1	0	0	0	0	0	0	0	0	0
	E	26	4	2	5	1	0	1	0	1	1	1	0

^aThe number of days of collection are different for each year. Therefore the columns cannot be directly compared.

^bIndicates number of days of collecting.

^cControl.

^dExposure.

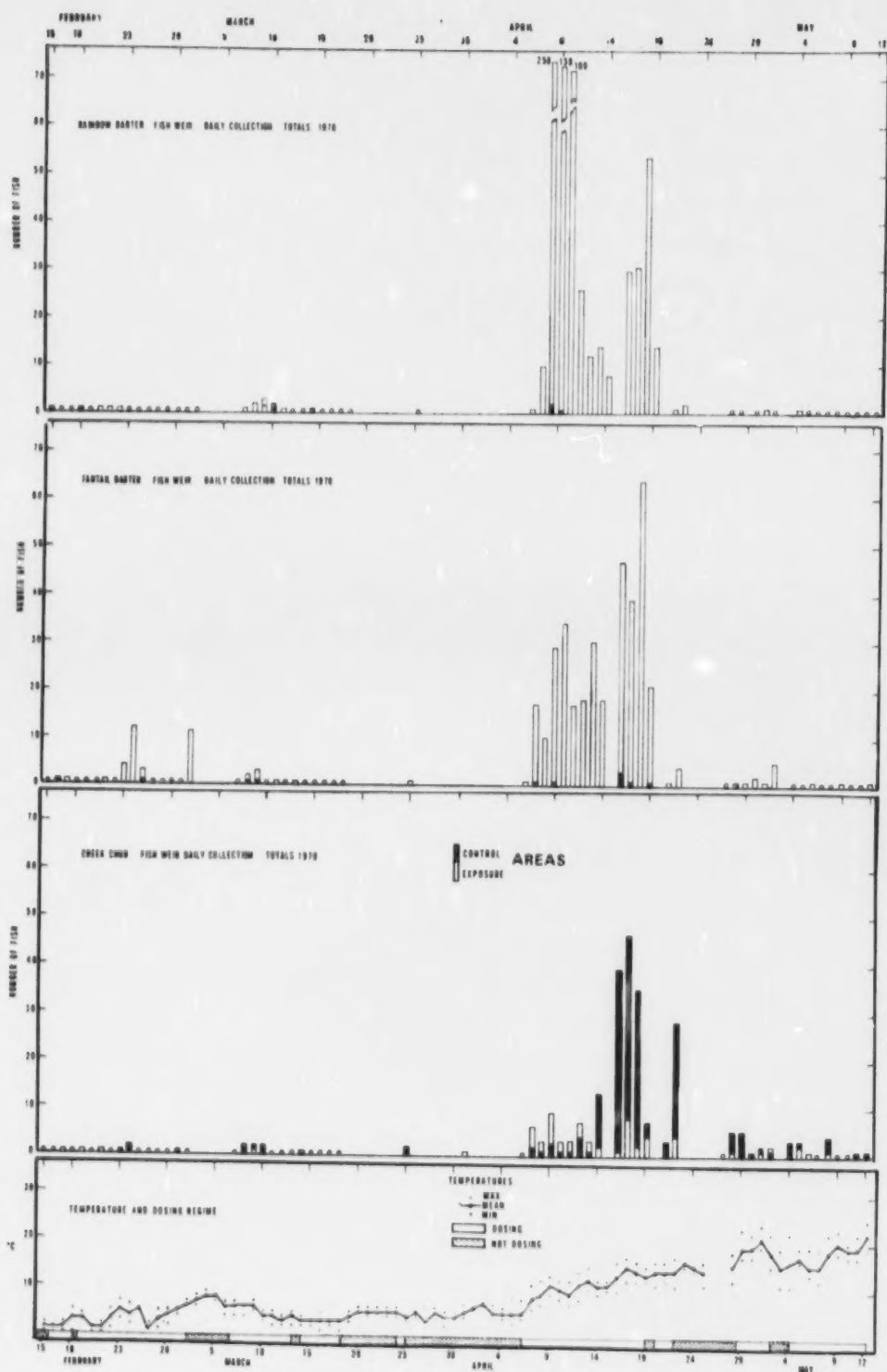


Figure 19. Daily collections of various fish species from the weir screens during 1970, and stream temperatures and dosing regimes at the time of collection.

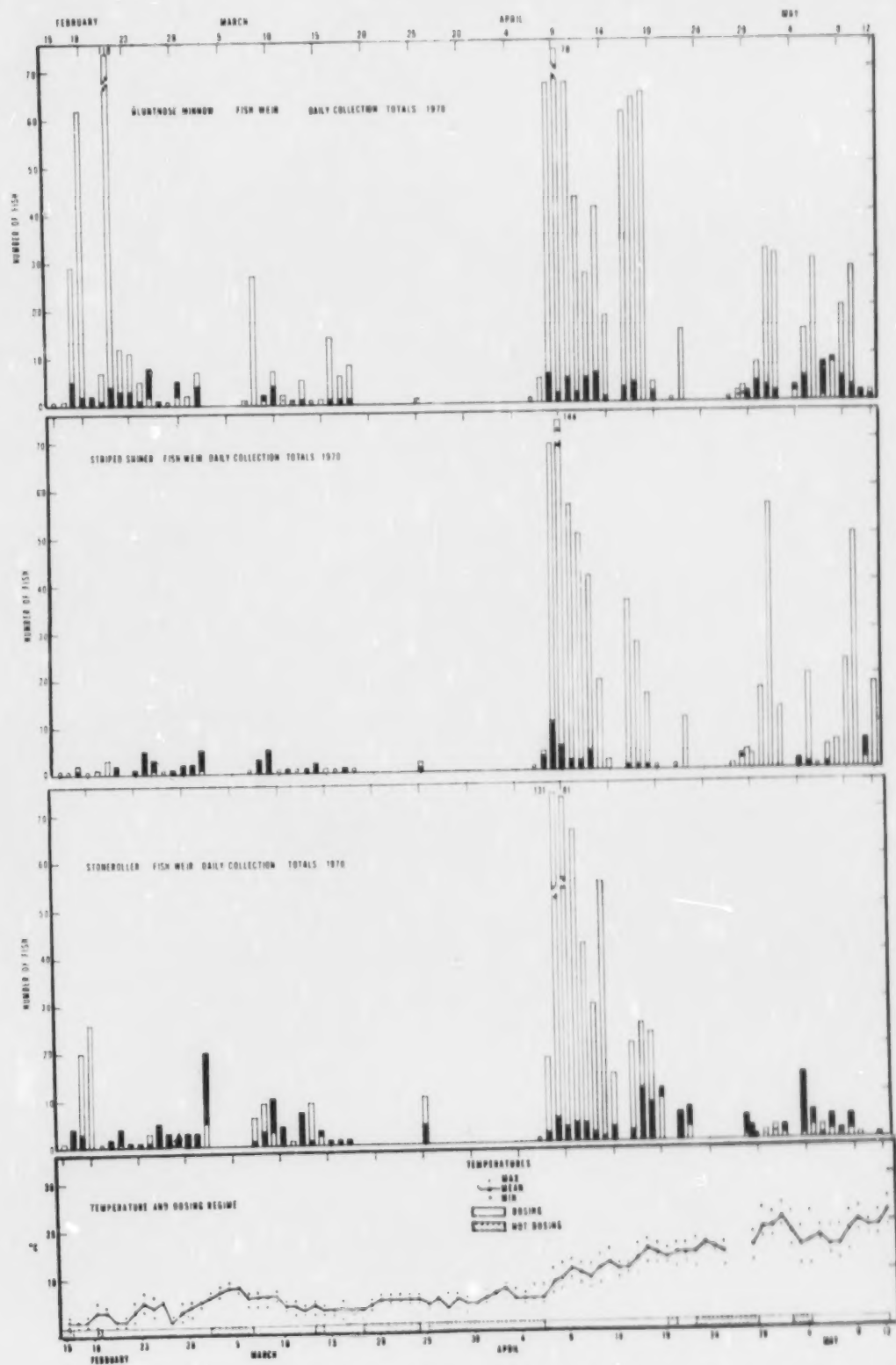


Figure 19. Daily collections of various fish species from the weir screens during 1970, and stream temperatures and dosing regimes at the time of collection.

TABLE 8. NUMBER OF YOUNG-OF-THE-YEAR ORANGETHROAT DARTERS
COLLECTED ON WEIR SCREENS, SHAYLER RUN, 1970-72

	Spring 1970	Spring 1971	Spring 1972
		<u>Control weir</u>	
Dosing	11 (53) ^a	10 (56) ^a	31 (21) ^a
Not dosing	0 (8)	11 (16)	21 (15)
		<u>Exposure weir</u>	
Dosing	38 (53)	89 (56)	293 (21)
Not dosing	4 (8)	56 (16)	221 (15)

^aNumber of collectings.

rainbow darter, and fantail darter, began a mass exodus from the exposure area on April 7 or 8, 1970. The increase in numbers of specimens captured on the exposure weir screen was correlated with an increase in water temperature. The average daily stream temperature increased from 5° C on April 6 to 11° C on April 9. The rainbow darter, fantail darter, stoneroller, and striped shiner were just starting their spawning activities at this time. It is believed that with increased spawning activity, plus the increased activity caused by copper exposure, more stress was placed on the population, and a behavioral change or avoidance reaction occurred.

To a limited degree creek chubs responded similarly. However, this was the only species for which more specimens were captured from the control weir than from the exposure weir. During the first week of exposure creek chubs gave no indication of downstream movement. From April 7 through 22 downstream movement of this species from the exposure area increased slightly (Figure 19). At the same time the bluntnose minnow, striped shiner, stoneroller, rainbow darter, and fantail darter were moving downstream, but the numbers of creek chubs were much lower. On April 14 a definite movement of creek chub downstream from the control area began. This movement was not exhibited by any of the other stream fish species. The creek chub is a high gradient stream species and is normally associated with the uppermost headwaters (Breder and Rosen, 1966). It has a tendency to migrate downstream for spawning since favorable spawning sites for this species are more abundant in lower stream gradient. This would partially explain the capture on the control weir-screen of numerous individuals in advanced spawning conditions. These fish could easily be stripped of eggs or milt. During May a number of the specimens captured on the control weir-screen were "spent" after spawning and were damaged or had fungus infections, apparently from their aggressive spawning behavior. Copper probably did not cause major downstream movement by this species.

Spawning Observations

Thirteen species of fish were observed spawning during the pre-exposure period in both control and exposure areas of the stream. Six of these, bluntnose minnow, striped shiner, creek chub, stoneroller, green sunfish, and white sucker, were common in both control and exposure areas, and their spawning activities in the two areas were similar before copper exposure began. Longear sunfish were scarce in the control area and fairly abundant in the exposure area before the introduction of copper. The control area was probably a marginal habitat for this species.

Only 11 species were observed spawning in Shayler Run during the exposure period (Table 9). Fantail darters and rainbow darters were observed spawning in both control and exposure areas during pre-exposure, but not during the exposure period. Bluegill and largemouth bass are considered pond or lake species, and blacknose dace was not common in the exposure area. These three species, therefore, will not be considered in detail.

Stoneroller, striped shiner, and creek chub spawned abundantly throughout the study area before copper exposure. However, no spawnings were observed for these species in the exposure area during the exposure period (Table 9). The total number of spawnings observed in the control area during the exposure period was 66, 30, and 34 for the stoneroller, striped shiner, and creek chub, respectively.

TABLE 9. NUMBER OF FISH SPAWNINGS OBSERVED IN CONTROL AND EXPOSURE AREAS OF SHAYLER RUN DURING THREE SEASONS OF COPPER INTRODUCTION

Species ^a	1970		1971		1972		Total	
	Control area	Exposure area	Control area	Exposure area	Control area	Exposure area	Control area	Exposure area
Bluntnose minnow	26	1	22	1	0	1	48	3
Striped shiner	16	0	8	0	6	0	30	0
Creek chub	4	0	15	0	15	0	34	0
Stoneroller	6	0	47	0	13	0	66	0
Rainbow darter ^b								
Fantail darter ^b								
Orangethroat darter	2	2	7	2	3	2	12	4
Green sunfish	19	35	30	13	28	20	77	68
Longear sunfish	4	78	2	33	5	32	11	143
Blacknose dace	0	0	10	0	1	0	11	0
White sucker	0	0	16	3	0	0	16	3
Bluegill	27	5	0	0	2	0	29	5
Largemouth bass	4	0	0	0	0	0	4	0

^aAll of the listed species were observed to spawn throughout the control and exposure areas for the 2 years before copper was introduced, but the number of spawns were not recorded.

^bSpecies for which spawning was not observed during the exposure period.

During the 1970 spawning season prespawning activity was observed for each of the above three species in the exposure area. A group of stonerollers was observed cleaning a nesting site and displaying spawning activity at a point approximately 695 m below the V-notch weir, but no eggs were deposited. This activity was observed only once. Four groups of striped shiners displayed spawning activity in the exposure area at three locations. Two of these were observed approximately 25 m below the point of copper introduction; one group was observed at the downstream end of the exposure biannual fish-collecting pool, 200 m below the V-notch weir; and the other group was located 620 m below the V-notch weir (Figure 3). No eggs were found at any of the above locations, and the activity did not persist more than 2 days. Three large male creek chubs prepared and defended nests approximately 25 m downstream from the V-notch weir, but no females were ever observed with them and the nest had no eggs.

During the 1972 spawning period a limited number of spawning observations were made in the recovery area between the downstream fish weir and the East Fork, Little Miami River. Striped shiners were observed spawning in this area on four occasions. The most upstream point of their spawning was approximately 1,340 m downstream from the V-notch weir, where concentrations of copper for the period ranged from 62 to 69 $\mu\text{g}/\text{L}$. Stonerollers were observed spawning on two occasions at a point 2,550 m downstream from the V-notch weir in the recovery area, where the copper concentration ranged from 40 to 53 $\mu\text{g}/\text{L}$. Creek chubs, however, were not observed spawning in the recovery area in 1972, even though observations were made during their most active spawning period.

Bluntnose minnow spawned well in the control area, but spawning was very limited in the exposure area during the exposure period. There were 48 spawnings in the control area but only three in the exposure area (Table 9). No spawnings were observed in the control during the 1972 season because of heavy rains and turbid water. Numerous fry of this species were observed in the control area in 1972, however, indicating that spawning had occurred. The three spawnings that were observed in the exposure stretch during the three seasons were located at the extreme downstream portion, where the copper concentration during the spawning period averaged 60 $\mu\text{g}/\text{L}$ and ranged from 35 to 77 $\mu\text{g}/\text{L}$. The numbers of eggs in these three spawns ranged between 200 and 250 and were probably the result of a limited number of females. In contrast, the numbers of eggs observed in the average control area spawnings were from 1,500 to 3,000. During the 1970 spawning season (on three occasions) bluntnose minnow males were observed guarding nesting sites in the exposure pool; no eggs were found at these locations, and the males left after a few days.

Heavy rains and turbid waters during April 1970 and 1972 prevented spawning observations of white suckers, but during 1971 they were observed spawning in the control area 16 times (Table 9). In the exposure area only three spawnings were observed for this species. These three spawnings were confined to the extreme downstream portion, where the average copper concentration during this period was 99 $\mu\text{g}/\text{L}$. These spawning sites were poor for white suckers. The nests were against the exposure weir stop logs, in water depths of 0.45-0.6 m, and the bottom was composed of silt, sand, and leaf detritus. In contrast, white sucker spawning sites in the control area, which are considered good spawning habitats, consisted of a fine gravel and fragmented limestone bottom substrate and flowing water of 0.3 m or less in depth. Habitats of this type were available in the exposure area, but were not used. The spawn from these exposure area nests developed to an advanced embryonic stage, but did not hatch. Failure of hatching is not attributed

to copper, but probably to inadequate water circulation around the eggs and thus insufficient oxygen.

During the two pre-exposure spawning seasons, green sunfish spawned in all the pools in both the control and exposure areas. Likewise, longear sunfish spawned in all the exposure area pools, but spawning of this species in the control area was limited and the population was usually low (Table 4). For the three spawning seasons during the exposure period, green sunfish spawned well in both the control and exposure areas, 77 and 68 spawnings, respectively (Table 9). The longear sunfish during this same period had limited spawnings in the control area, as was expected. There were 11 compared to 143 in the exposure area. Adult individuals of both species were present throughout the exposure area during their spawning seasons. Spawning of these sunfish species was restricted, however, to the downstream half of the exposure area, where the average copper concentration during the spawning season was $90 \mu\text{g/L}$ or less. During the first year of exposure, prespawning activity was observed for both green sunfish and longear sunfish in the exposure pool (Figure 3), which is in the upstream half of the exposure area where the average copper concentration is greater than $90 \mu\text{g/L}$. This activity consisted of male individuals cleaning and guarding nesting sites. No females were observed in spawning activity on these sites, however, nor were eggs found. This activity lasted only a few days, and the nests were abandoned. Restriction of spawning of green and longear sunfish to the lower half of the exposure area reduced the production potential for young based on area alone, and more than likely reduced their total production in the exposure area.

Orangethroat darters were observed spawning throughout the control and exposure areas during the three spawning seasons of exposure. Their spawning was not restricted by copper concentration in the exposure area. Much more spawning activity was observed than was confirmed by actual observation of eggs. The eggs of this species were exceedingly hard to find, since they were buried in fine gravel and sand.

Fry Collections

Many of the fry data obtained from the 1970 fish-weir collections were estimates, especially for the control weir (Table 10). Because the specimens were in poor condition, only white sucker fry were identifiable. Therefore, the collections only show the effect of copper on total number of fry and white sucker fry captured on the exposure weir.

Eight times as many white suckers were captured on the control weir as on the exposure weir, 5,768 and 764, respectively. The weirs were flooded on April 23, 1970, for a 72-hr period and again on May 2, 1970, for 23 hr. White suckers had spawned in the control area before these dates, and white sucker fry were observed only in the control area on April 29, 1970. Thus, it is likely that the white suckers captured in the exposure weir were produced in the control area and were washed downstream during the flooding. The white sucker data as well as the reduced total numbers of fry captured on the exposure weir compared to the control weir, 858 versus 11,088, respectively, indicated that spawning in the exposure area was adversely affected by copper. The situation was repeated in 1971 (Table 11).

The fry data presented for 1971 are actual values (Table 11). The specimens were separated into the following categories: white sucker, sunfish, bluntnose

TABLE 10. NUMBER OF FRY COLLECTED ON WEIR SCREENS IN SHAYLER RUN, 1970

Date	Control screen			Exposure screen			Remarks
	White number	Unidentified fry	Total	White number	Unidentified fry	Total	
4/22							Weir flooded: 4/23
4/29	60		60				
4/30	600		600				
5/1	3,860		3,860	200		200	
5/4	300		300	125		125	Weir flooded: 5/2
5/5	22	4	26	15	23	64	
5/6	180		180	21		21	
5/7	31		31				
5/8	628		628	68		68	
5/11	5	495	500	300		300	
5/18		1,000	1,000		5	5	
5/19	20	980	1,000		4	4	
5/21	30	1,170	1,200		8	8	
5/22		1,100	1,100		10	10	
5/29		302	302				
6/10		27	27				
6/12		29	29				Weir flooded: 7/8
6/29	12	13	25	11	22 ^a	31	Weir flooded: 8/7
7/13							Weir flooded: 8/20
9/9							
Totals	5,266	5,420	11,088	764	94	858	

^aMT moltbeat fry.

TABLE 11. SPECIES AND NUMBERS OF FRY COLLECTED ON WEIR SCREENS IN SHAYLER RUN, 1971

	Control screen						Exposure screen							
	White sucker	Sunfish	Bluntnose minnow	Darter	Bullhead	Unidentified fry	Total	White sucker	Sunfish	Bluntnose minnow	Darter	Bullhead	Unidentified fry	Total
1971							0							0
5/5 ^a							2							0
5/11	2						0							0
5/17							0							0
5/24	9	5				4	18	2						2
5/28 ^b	92					6	98	2			2			4
6/2	3					1	4							0
6/4	15						15							0
6/7	265			40			305	5						5
6/11	11	3		2		5	21		1					1
6/13	5	192				10	207		64					64
6/18		3,994					3,994	10	440	30				480
6/21		1,820	60				1,880		120	60				180
6/25		3,260	270		20		3,550		1,751	60				1,811
6/28		155					155		764			2		766
7/15		1,650	1,760				3,410		1,160	20				1,180
7/19		318	128				446		569					569
8/6		13				1	14		120					120
8/30							0		10					10
Species total	402	11,410	2,218	42	20	27	14,119	19	4,999	170	2	2	0	5,192

^a5/6 and 5/7 - Weir flooded.

^b5/25 - Weir flooded.

minnow, darter, bullhead, and unidentified fry, the latter probably composed of creek chub, striped shiner, stoneroller, and blacknose dace. The fry collections from control and exposure fish weirs, in 1971, 11, 410 and 4,999, respectively, were dominated by sunfish. The control sunfish were predominantly green sunfish rather than longear sunfish based on the difference in the number of spawns observed for the two species (Table 9). It is apparent that in 1971, when fry specimens were in good condition and could be identified, unidentified fry were not very abundant in the weir collections--only 27 specimens in the control and none in the exposure. Evidently the species represented by these fry did not move downstream during normal stream flow ($2.55 \text{ m}^3/\text{sec}$ or less) as did the white sucker, sunfish, and bluntnose minnow fry.

In 1971 white sucker fry were first observed in the control area on May 4; they were abundant on May 5. No fry of this species had been observed in the exposure area before or on these dates. The fish weirs were flooded on May 6 for 7 hr and again on May 7 and 8 for another 42 hr. White sucker fry were first observed in the exposure area on May 10.

Bluntnose minnow fry were first observed in the control area in 1971 on May 23, but none were observed in the exposure area before or on this date. On May 25 the weirs were flooded. On May 29 the first bluntnose minnow fry were observed in the exposure area. Thus, bluntnose minnows were probably produced in the control area and were washed downstream during flooding of the weir.

Sunfish fry were not exposed to high water and flooding of the weirs during the 1971 spawning period. Reproductive success of this species in the control and exposure areas is accurately indicated by the weir-screen data. Spawning success of sunfish in the exposure area was not drastically affected by the addition of copper.

In 1971 fry traps captured fry of 12 species (Table 12). Traps 1, 2, and 3 are control-area traps, traps 4, 5, and 6 are exposure-area traps (Figure 3). The fantail darter occurred in large numbers in both the control and exposure stretches, but only three fry were captured by these traps.

In the control area bluntnose minnows, striped shiners, creek chubs, stonerollers, orangethroat darters, blacknose dace, and white suckers spawned successfully, and their young were well represented in the fry-trap collections. Although rainbow darters and johnny darters were not observed spawning, sufficient numbers of these species were taken in the traps to indicate that spawning had occurred. Longear sunfish fry were not collected, but the adult population was low and only limited spawning of this species was observed in the control area in 1971. Green sunfish, on the other hand, spawned abundantly in the control area, but their young were not well represented in the trap collections.

In the exposure area only three species of fish were known to have spawned successfully: the green sunfish, longear sunfish, and orangethroat darter. They were well represented in the fry-trap collections. Spawning of bluntnose minnows was observed in the exposure area only once in 1971. Few fry of this species were taken in the exposure area traps when compared to the number captured in the control area. The majority, 22 of 32, were captured in trap 4, which is approximately 550 m upstream from the only observed spawning location (station 6 near the downstream fish weir) for this species. Probably most of the bluntnose minnows and all of the creek chubs, rainbow darters, blacknose dace, johnny

TABLE 12. SPECIES AND NUMBERS OF FRY COLLECTED IN FRY TRAPS IN SHAYLER RUN, 1971^a

Species	Control traps			Exposure traps		
	#1	#2	#3	#4	#5	#6
Bluntnose minnow	456	619	851	22	2	8
Striped shiner	41		100			
Creek chub	267	177	105	2	4	5
Stoneroller	20	12	11			
Rainbow darter	13	12	6	4	1	
Fantail darter				2		1
Orangethroat darter	39	12	24	16	5	16
Green sunfish		2	5	6	4	50
Longear sunfish				21	31	66
Johnny darter	31	10	19		1	5
Blacknose dace	31	49	57	1		
White sucker	192	27	133	1	52	81
Total for all species	1,090	920	1,313	75	100	232
			3,323			407

^aTwenty-two sampling periods, 5/19-8/30, 1971.

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darters, and white suckers captured in the exposure area were spawned in the control area and were transported to the exposure area during flooding of the fish weirs on May 6, 7, 8, and 25, 1971. All of these species were present in the control area before flooding, but they were not observed in the exposure area until after flooding. No striped shiners or stonerollers were taken in traps in the exposure area during this period. Seven times more fry were captured in the control area than in the exposure area, indicating that the addition of copper caused a reduction on reproduction in the exposure area.

SECTION XI

EFFECTS OF COPPER ON STREAM BENTHIC COMMUNITIES

INTRODUCTION

The purposes of this portion of the study were to investigate the effects of copper on the macroinvertebrates of the stream and to relate, if possible, these effects to the feeding habits of the stream fish through analysis of stomach contents. In addition, sampling was done from July 17, 1968, through June 14, 1971, to evaluate the effects of copper on the periphyton community.

During the early pre-exposure period, between May 1968 and February 1969, various apparatus and methods for collecting qualitative and quantitative macroinvertebrate samples were tried to determine baseline population levels. Since the stream riffles are composed of large limestone rubble and the bottom of the pools is bedrock, conventional samplers, such as the Ekman or Petersen dredge, Surber sampler, or multiplate sampler, could not be used. The basket sampler was the most efficient sampler tested and was used to collect macroinvertebrates during the pre-exposure period from March 7, 1969, to February 16, 1970.

During the exposure period macroinvertebrates were collected from February 16, 1970, through July 15, 1971. Samples were taken from (1) artificial substrates, created by suspending rock-filled baskets; (2) the natural substrate; and (3) weir screens in both the control and exposure areas.

METHODS

Basket Samplers

Basket samplers used for collecting macroinvertebrates were the same as those described by Mason *et al.* (1967, 1970). They were filled with 36 limestone rocks, 6.5-9.5 cm in diameter. The baskets were permanently attached to specially designed "T's" made of black iron pipe, which were inserted over iron stakes driven into the stream bottom (Figure 20). When in position approximately 9 cm above the bottom, the baskets were always directed downstream and could move freely so that silt and sand would not accumulate in or around them, and they were always covered with water. Paired samplers were positioned approximately 75 cm apart in pools immediately downstream from riffles at stations located in midstream, 50 cm above copper introduction; in the upper exposure area, 160 m downstream from copper introduction; in the lower exposure area, 815 m downstream from copper introduction; and in the recovery area, 2 km downstream from copper introduction (Figure 3).

Basket samplers were left in place for 6-week periods and were removed as close to the end of these periods as stream and weather conditions permitted.

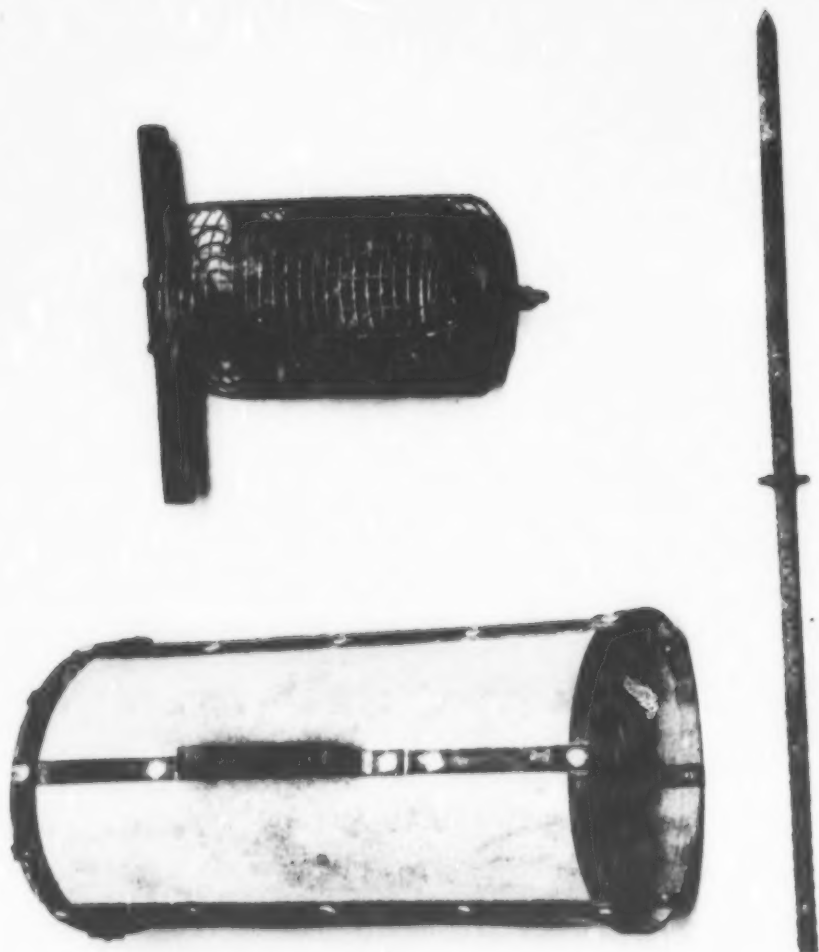


Figure 20. Rock-filled basket sampler for collecting macroinvertebrates and the wire-mesh screen used to cover sampler when removing it from the stream.

They were removed from the stream with the aid of a cylinder made of U. S. standard number 30 wire mesh (50cm long, 25 cm ID), which fitted over the baskets to prevent loss of organisms (Figure 20). The rocks were dumped into a tub partially filled with stream water and scrubbed with a stiff brush to remove organisms. The samples were concentrated in a U. S. standard number 30 sieve (0.59-mm mesh opening), preserved with 70% ethanol in plastic containers, and stored in the laboratory for later sorting and identification. The cleaned samplers were then reset at the same locations.

Natural Substrate Sampling

Natural substrate sampling was done in a control riffle immediately downstream from the control pool, 275 m upstream from copper introduction; in the two exposure riffles, 200 m downstream from copper introduction and 720 m downstream from copper introduction; and in a recovery riffle, 2.2 km downstream from copper introduction (Figure 3). Natural substrate samplings were usually collected on the same day that basket samplers were removed.

Rocks were selected randomly for 10 min, were placed in a tub partially filled with water, and were scrubbed clean with a stiff brush to remove the organisms. These samples were processed in the manner previously described for artificial substrate samples.

Weir-Screen Collections

Samples were collected from the control and exposure weir screens on the days when flow was 0.25 m³/sec or less and copper was being added. The material containing macroinvertebrates was gently hosed from the screens with water into the weir troughs and collected in the wire-mesh baskets (0.5-mm openings) at the ends of the troughs. This material was then put in 1-gal jars and immediately preserved in 7% formalin. The samples were thoroughly washed in tap water in the laboratory and stained with a solution of 200 mg/l. rose bengal in 70% ethanol. These samples contained large volumes of leaves, stems, bud scales, and seed pods, and staining improved the thoroughness and rate of sorting the organisms (Mason and Yevich, 1967).

An additional technique was used to separate macroinvertebrates more effectively from debris. Small portions of the sample were washed with tap water in a U. S. standard number 30 sieve to remove excess stain and were placed on a strip of fine mesh wire screen that was then placed in a shallow white porcelain pan containing a small amount of water. This created a contrasting background against which the stained organisms could easily be seen and picked from the debris. When samples contained excessive debris, aliquots were examined.

All specimens from the various sampling techniques were identified to family. Organisms found in large numbers were identified to genus and species in most cases. Macroinvertebrates collected by all the techniques are recorded as numbers per sample.

Macroinvertebrate samples were collected by hand and dip nets on May 28 and June 13, 1969, by William R. Mason, Jr., and Phillip A. Lewis of the Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio, (formerly known as the Analytical Quality Control Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio). The organisms were

identified and sent to this laboratory to used as a reference collection (Table 13). Specimens of amphipods were identified by Dr. John R. Holsinger of Old Dominion University, Norfolk, Virginia. His identifications were corroborated by Dr. E. L. Bousfield of the National Museum of Natural Sciences, Ottawa, Ontario.

In early summer 1970 a copper-concentration gradient became evident in the exposure and recovery areas when the water temperature had increased. Additional information could be gained in this gradient, so additional natural substrate stations were established in the lower exposure and recovery areas. Basket samplers in the recovery area in June 1970 were destroyed by vandalism and replaced, and the first collection from this area was made in late August.

Periphyton samples were collected for approximately 3 years from natural and artificial substrates in both riffle and pool in the control and exposure areas. The control riffle and pool were 350 and 275 m, respectively, upstream from the point of copper introduction; the exposure pool and riffle were 410 and 200 m, respectively, downstream from the point of copper introduction. The parameters examined included cell density, dry weight, ash-free weight, chlorophyll content, biomass-to-chlorophyll a ratio, pheophytin-to-chlorophyll a ratio, species composition, and species diversity.

RESULTS AND DISCUSSION

Pre-exposure macroinvertebrate samples from the control and exposure areas contained organisms representing 15 and 14 orders, respectively. These were composed of sufficient numbers of taxa to indicate that the test area had recovered from any deleterious effects of the Shayler Run Waste Treatment Plant on the stream. The species list presents all of the organisms collected and identified from all of the sampling procedures used during the study (Table 13).

The number of macroinvertebrates collected in each basket of the paired samplers at three locations in June and July is shown in Table 14. The data from the paired samplers were combined for evaluation of the macroinvertebrate populations at each station and are recorded as numbers per station (Table 15). Voids in these data are the result of vandalism, flood damage, or poor preservation.

Natural substrate sampling yielded organisms that were not normally collected by basket samplers and provided additional information for evaluation of the macroinvertebrate population. Macroinvertebrate organisms collected from weir screens were those that were transported by stream currents. This was an excellent sampling of classical drift organisms, since the screens collected organisms from the entire stream flow.

The predominant groups of macroinvertebrates collected from Shayler Run, Isopoda, Ephemeroptera, Amphipoda, Chironomidae, Psephenidae, and Tricoptera, are dealt with in detail below. The other groups shown in the tables represent too small a percentage of organisms to warrant further discussion.

Isopoda (sowbugs)

Only one species, Lirceus fontinalis, was present in the stream. Basket-sampler collections showed that the population of sowbugs in the exposure area during the pre-exposure period was as great as, if not greater than, it was

TABLE 13. SPECIES OF MACROINVERTEBRATES COLLECTED BY ALL METHODS IN SHAYLER RUN DURING 1969-71

Organisms	Collection areas											
	Control			Upper exposure			Lower exposure			Recovery		
	'69	'70	'71	'69	'70	'71	'69	'70	'71	'69	'70	'71
Diptera (True Flies)												
Chironomidae (Midges)												
Ablabesmyia sp. 1		X	X	X	X	X			X		X	X
Calopsectra glabrescens	X	X	X	X	X	X			X		X	X
C. (pess.) macromandatum (Kieff)	X	X	X	X	X	X	X					
Chironomus attenuatus Walker	X	X	X	X	X	X		X	X			X
C. plumosus Linnaeus		X		X	X							
C. riparius Gr.	X			X			X					
Conchapelopia sp.	X											
Coryneura (Thienemanniella) Xena	X			X								
Cricotopus bicinctus gr.	X	X		X	X				X			
C. setis	X		X									
C. slovicus		X	X	X								
C. trifasciatus		X	X	X								
Cryptochironomus argus	X	X		X								
Damesa longimanus							X					
Glyptotendipes lobatulus (Say)	X	X		X								
Metricnemus sp.	X	X	X	X								
Orthocladius nivorundus	X	X		X	X							
O. scutellatus	X	X		X								
Paratendipes sp.	X			X								
Pentaneura flavifrons (Joh.)	X	X	X	X	X	X	X	X	X	X	X	X
P. mobilis	X			X	X		X					
Polypedilum convictus	X	X	X							X		
P. tritum	X	X		X	X	X	X					
Pseudocricotopus riparius	X	X		X	X	X	X					
Pseudocricotopus sp.	X	X	X	X			X					
Tanytarsus (Paratanytarsus) sp.	X	X	X	X	X		X					
T. (Cladotanytarsus) sp.	X			X								
T. (Cladotanytarsus) sp. 3 Roback	X			X								
Tribolus sp.		X								X	X	
Ceratopogonidae (Biting Midges)												
Atrichopogon sp.		X			X							
Bezzia varicolor		X	X		X							
Probezzia glabra (Coq.)		X										
Chaoboridae (Phantom Midges)												
Chaoborus sp.		X	X		X	X						
Culicidae (Mosquitoes)		X			X							
Simuliidae (Blackflies)												
Simulium sp.	X	X	X	X	X	X	X	X	X	X	X	X
Tabanidae (Horseflies)					X							
Stratiomyidae (Soldierflies)												
Euperyphus greylackensis		X			X							
Odonotomys cincta	X		X									
Stratiomys sp.					X							
Tipulidae (Crane Flies)												
Antocha saxicola		X	X		X							
Megastocera longipennis		X	X		X							
Tipula sp.		X	X		X	X						
Psychodidae (Moth Flies)												
Psychoda alternata (Say)	X			X			X					
Ephemeroptera (Mayflies)												
Baetidae												
Baetis sp.	X	X	X				X					
Neotoma alenense	X	X		X			X					
Ephemeridae												
Hesagenia limbata		X	X		X	X		X	X			
Caenidae												
Caenis sp.	X	X	X	X	X	X	X	X	X			
Heptageniidae												
Stenonema (Femoratum) scitulum	X	X	X	X	X	X	X	X	X	X	X	X
S. intermedium	X	X	X	X	X	X	X	X	X	X	X	X
S. tripunctatum	X	X	X	X	X	X	X	X	X	X	X	X
Colopectera (Beetles)												
Hydrophilidae (Predaceous Water Beetles)												
Deronectes sp.		X			X							
Leucophaea terminalis		X										
Elmidae (Riffle Beetles)												
Nephus sp.			X									
Neelmis sp.					X							
Optoserous sp.	X	X	X	X	X	X						
Prometis elegans												
Stenelmis lateralis	X	X	X	X	X	X	X	X	X	X	X	X
S. marshalli	X	X	X	X	X	X						
Talitrea sp.			X		X							
Gyrinidae (Whirligig Beetles)					X							
Malpighiidae (Crawling Water Beetles)												
Peltodytes simplex		X	X		X	X						
Hydrophilidae (Water Scavenger Beetles)												
Enochrus sp.		X	X									
Hydrochus sp.		X										
Psephenidae (Water Penny)												
Psephenus herricki	X	X	X	X	X	X	X	X	X	X	X	X

TABLE 13 (continued). SPECIES OF MACROINVERTEBRATES COLLECTED BY ALL METHODS IN SHAYLER RUN DURING 1969-71

TABLE 14. NUMBERS OF MACROINVERTEBRATES COLLECTED IN JUNE AND JULY FROM PAIRED (A and B) ROCK-FILLED BASKET SAMPLERS IN SHAYLER RUN, 1969-71

DATE	AREA	Amphipoda (number)	Amphipoda (% total)	Isopoda (number)	Isopoda (% total)	Hydracarina (number)	Hydracarina (% total)	Collembola (number)	Collembola (% total)	Rotifera (number)	Rotifera (% total)	Planarians (number)	Planarians (% total)	Other (number)	Other (% total)	SAMPLER TOTAL	PERCENT TOTAL	TOTAL TOTAL
1969																		
6/8	Control A	1,089	20	37	13	2	987	0	2	36	20					5,391		
	Control B	1,153	10	42	31	1	989	0	0	40	21					1,280		1,479
	Upper Exposure A	6,901	12	17	200	116	405	17	6	21	300					6,829		
	Upper Exposure B	5,395	4	20	118	20	264	2	0	21	171					5,596	100	11,941
	Lower Exposure A	1,123	8	16	35	1	5	2	5	12	20					1,490		
	Lower Exposure B	1,721	15	49	48	5	34	20	2	127	62					1,218		1,908
7/1	Control A	31	0	10	140	16	23	2	1							191	111	216
	Control B	4	0	5	291	19	20	1	1	1	1					118	111	236
	Upper Exposure A	22	0	18	403	9	4	4	2	4	10					513	111	560
	Upper Exposure B	67	0	58	312	7	31	30	1	2	11					513	111	560
	Lower Exposure A	65	0	1	50	22	31	18	1	0	196					411	100	562
	Lower Exposure B	93	0	22	350	18	30	15	8	0	7					477	100	562
LOWER EXPOSURE (JANUARY 2, 1970)																		
1970																		
6/1	Control A	907	0	8	30	0	74	0	1	0	1					983		
	Control B	506	0	7	21	0	71	0	0	1	0					61		1,221
	Upper Exposure A	14	0	1	4	0	11	0	2	0	0					27		
	Upper Exposure B	6	0	1	5	0	19	0	0	0	0					26		54
	Lower Exposure A	1	0	1	8	0	100	0	0	1	0					112		
	Lower Exposure B	7	0	0	0	0	10	0	1	4	0					18		113
7/14	Control A	2	0	2	148	0	18	0	0	0	0					173		
	Control B	0	0	1	133	0	32	0	0	0	0					176		349
	Upper Exposure A	0	0	0	1	1	8	0	0	0	0					11		
	Upper Exposure B	0	0	0	1	2	13	0	0	0	0					16		35
	Lower Exposure A	1	0	0	0	0	0	0	1	0	0					10		
	Lower Exposure B	0	0	0	0	0	0	0	1	0	0					11		46
1971																		
6/1	Control A	123	0	14	38	0	80	2	4	1	0					150		
	Control B	140	0	11	21	0	75	2	0	0	0					248		398
	Upper Exposure A	7	0	0	0	0	41	0	0	0	0					106		
	Upper Exposure B	5	0	0	0	0	11	0	0	0	0					128		234
	Lower Exposure A	0	0	0	1	0	60	1	1	0	0					62		
	Lower Exposure B	0	0	0	0	0	48	1	2	0	0					51		189
7/13	Control A	1	0	1	63	0	7	16	1	0	0					80		
	Control B	0	0	0	44	0	8	2	0	0	0					54		134
	Upper Exposure A	0	0	2	1	0	31	10	1	0	0					38		
	Upper Exposure B	0	0	2	0	1	12	8	1	0	0					23		71
	Lower Exposure A	0	0	2	30	0	11	8	1	0	0					54		
	Lower Exposure B	0	0	1	26	0	9	0	1	0	0					36		107

7

67

in the control area (Table 15, Figure 21). During the exposure period sowbugs were practically eliminated from both the exposure and recovery areas (Figures 21 and 22), whereas the population was maintained in the control area.

The weir screens were sampled often during the first year of exposure (Table 16, Figure 23); all the sowbugs captured were medium to large. The drift rate of sowbugs from the exposure area was nearly three times greater than it was from the control area during the period of February 23 to April 8, 1970. This was expected since a greater population was present. However, the exposure drift rate declined to a level lower than that of the control by April 22, 1970, as the population in the exposure area became depleted (Table 15).

No young and only a few adult sowbugs were collected in the exposure and recovery areas after June 1970, indicating that those animals that were transported downstream during high water did not survive in either exposure or recovery area. Further support for this conclusion is indicated by a test conducted during April and May 1971, in which 40 mature sowbugs were placed in each of two chambers at the streamside laboratory. A continuous-flow system was used whereby one chamber received control water and the other received water with a nominal concentration of 120 $\mu\text{g/l}$. of copper. During this test newly hatched sowbugs died within 2 days in the exposure chamber, whereas newly hatched young in the control chamber were never observed dead. At the termination of the study no young sowbugs were found in the exposure chamber, but in the control chamber 1,298 young were present. Thus, reduction in the population of sowbugs in the exposure and recovery areas during the period of exposure is attributed to copper through increased drift rate and death of the young.

Ephemeroptera (mayflies)

Seven species of mayflies were present in the various collections (Table 13). Data from basket samplers (Table 15, Figure 24) indicate that during the pre-exposure period the mayfly population was as great in the exposure area as in the control area. For the first month of copper exposure, the drift rates from the control and exposure areas were low and similar, as evidenced by the number of mayflies captured on the weir screens (Table 16, Figure 25). No mayflies were taken on either screen in the collection on March 17, 1970. On April 8, 1970, the number of mayflies captured on the exposure screens increased considerably, 334 and 5 on the exposure and control screens, respectively. Rising water temperature may have increased the sensitivity of the organisms to copper, which could account for the increased drift rate. The mean daily stream temperature from March 17 through April 6 ranged between 3° and 5° C. By April 8 the mean daily stream temperature had increased to 9° C, and it increased progressively over the next few weeks to 14° C by April 22. On this date 33 mayflies were captured on the control screen and 21 on the exposure screen. For the remainder of the exposure period greater numbers were captured on the control screens in most cases. Evidently the number of mayflies present in the exposure area had been reduced because there were fewer in the exposure basket samplers and natural substrate samples as well (Tables 15 and 17, Figures 24 and 26). Reduction may have been a result of increased drift rate or number of deaths, although deaths were not noticeable.

Peak population levels of mayflies occur normally in the summer and early fall. The low copper concentrations at the sampling stations in the recovery area probably permitted reproduction and survival of mayflies, whereas the copper



Figure 21. Isopoda (sowbugs): basket-sampler collections.

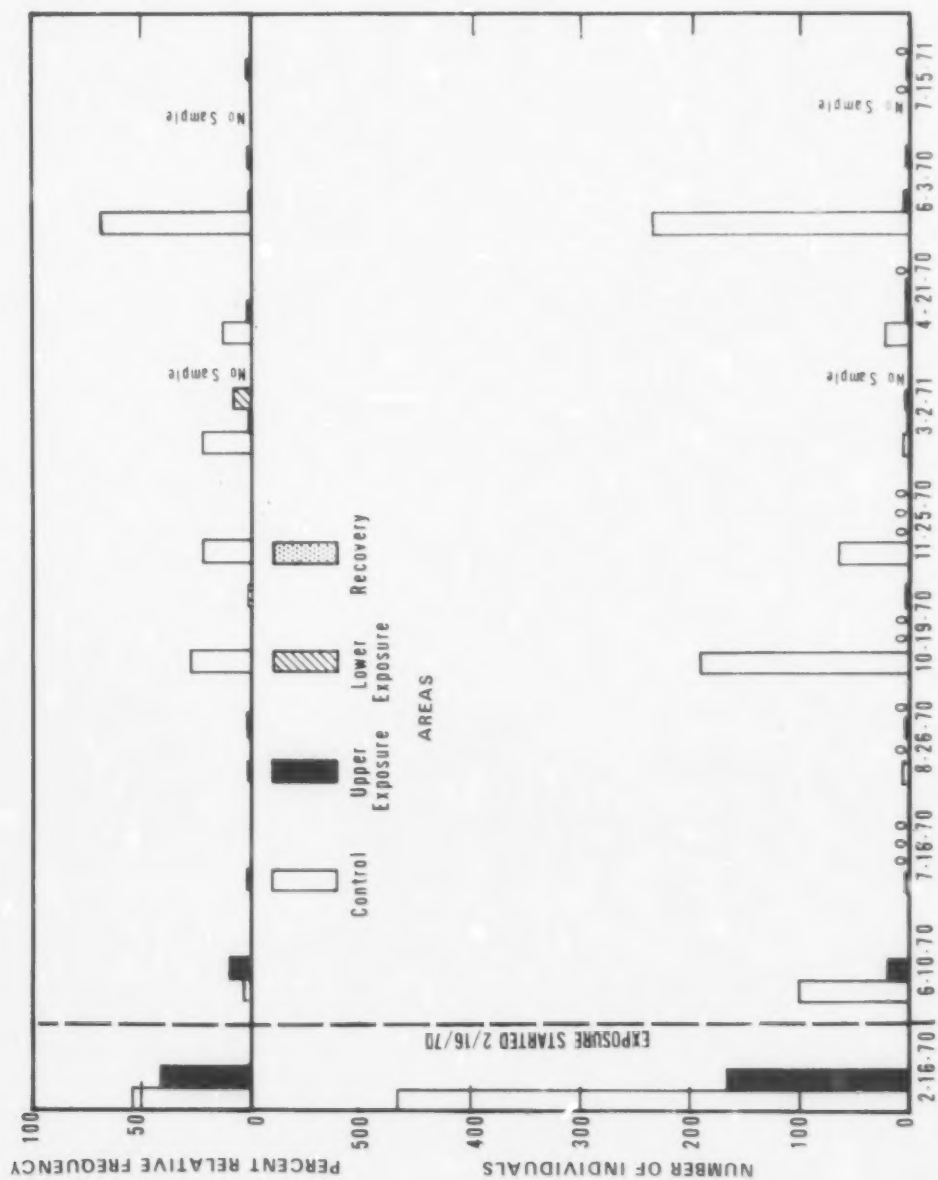


Figure 22. Isopoda (sowbugs): natural substrate collections.

A

Year	1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099
1900	1901	1902	1903	1904	1905	1906	1907	1908	1909	1910	1911	1912	1913	1914	1915	1916	1917	1918	1919	1920	1921	1922	1923	1924	1925	1926	1927	1928	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945	1946	1947	1948	1949	1950	1951	1952	1953	1954	1955	1956	1957	1958	1959	1960	1961	1962	1963	1964	1965	1966	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	

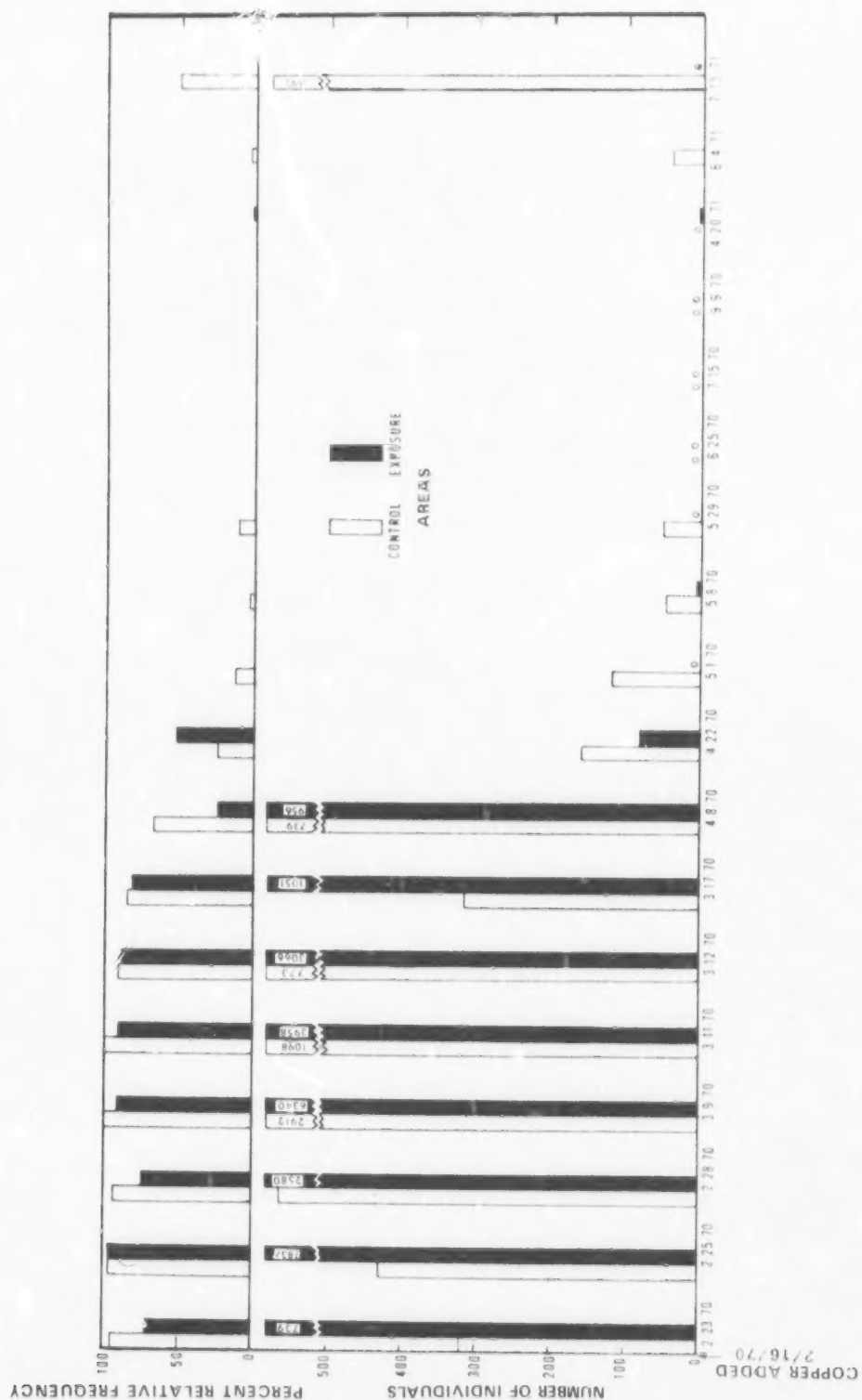


Figure 23. Isopoda (sowbugs): weir-screen collections.

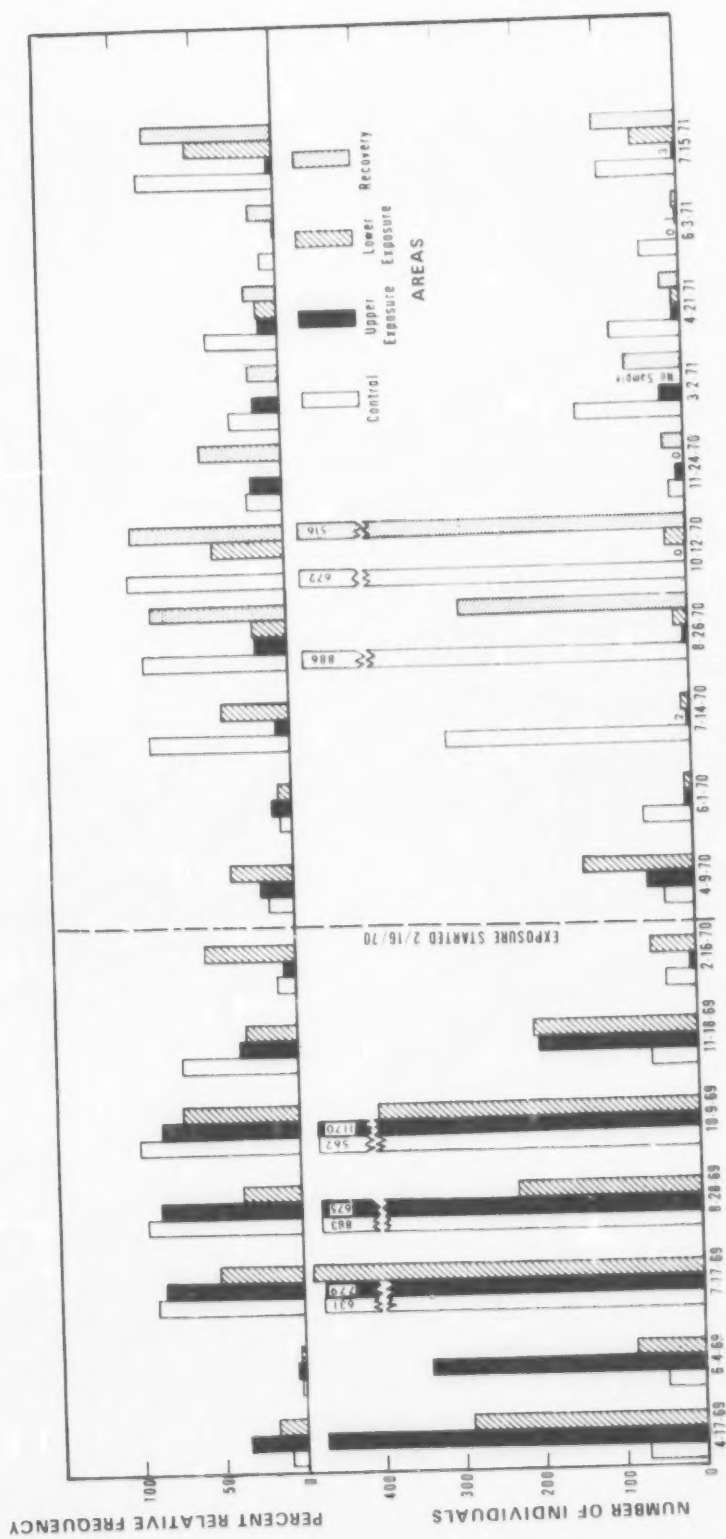


Figure 24. Ephemeroptera (mayflies): basket-sampler collections.

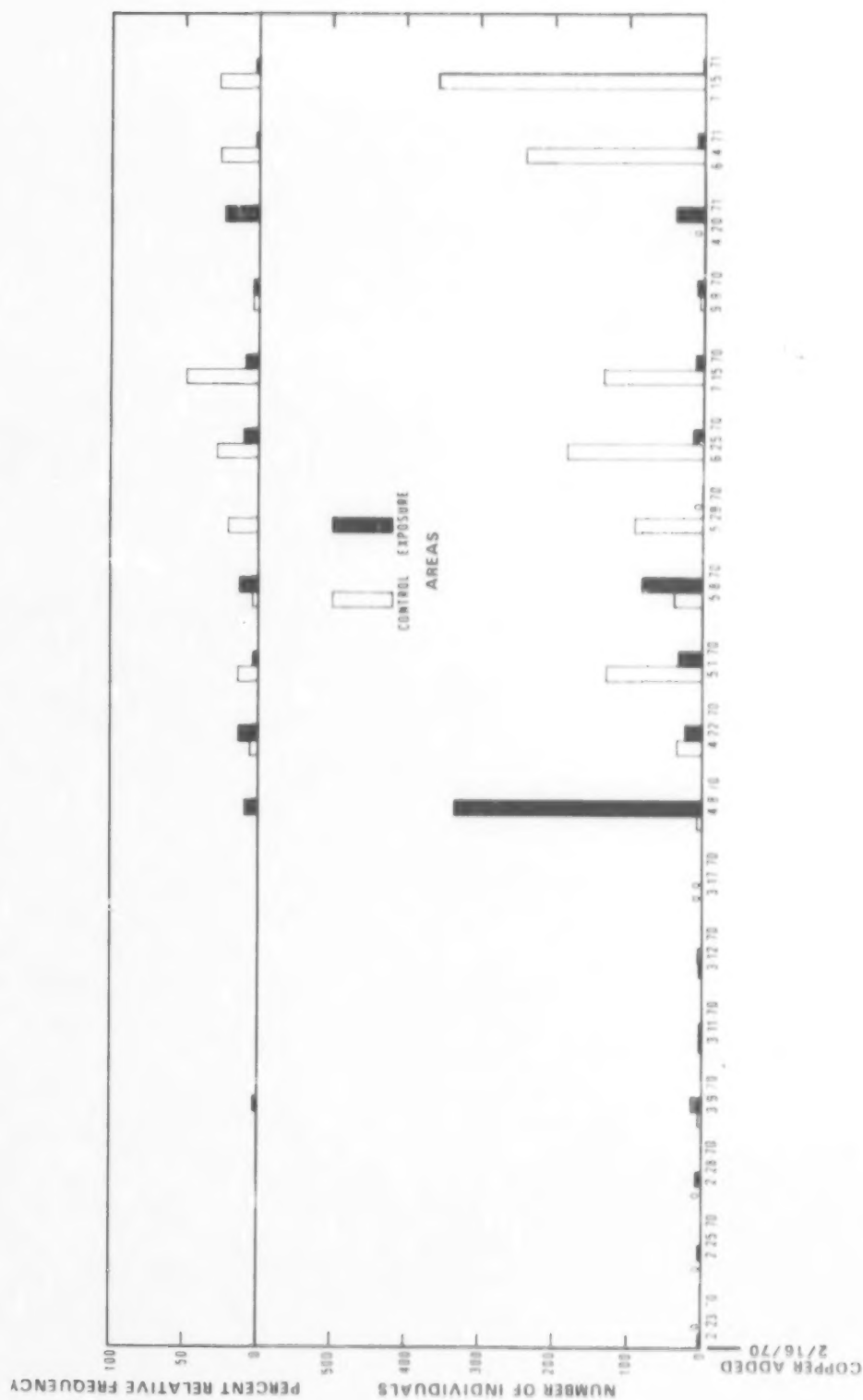


Figure 25. Ephemeroptera (mayflies): weir-screen collections.

TABLE 17. TOTAL MACROINVERTEBRATES COLLECTED FROM NATURAL SUBSTRATES
IN SHAYLER RUN DURING 1969-71

Station	Substrate	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2433	2434	2435	2436	2437	2438	2439	2440	2441	2442	2443	2444	2445	2446	2447	2448	2449	2450	2451	2452	2453	2454	2455	2456	2457	2458	2459	2460	2461	2462	2463	2464	2465	2466	2467	2468	2469	2470	2471	2472	2473	2474	2475	2476	2477	2478	2479	2480	2481	2482	2483	2484	2485	2486	2487	2488	2489	2490	2491	2492	2493	2494	2495	2496	2497	2498	2499	2500	2501	2502	2503	2504	2505	2506	2507	2508	2509	2510	2511	2512	2513	2514	2515	2516	2517	2518	2519	2520	2521	2522	2523	2524	2525	2526	2527	2528	2529	2530	2531	2532	2533	2534	2535	2536	2537	2538	2539	2540	2541	2542	2543	2544	2545	2546	2547	2548	2549	2550	2551	2552	2553	2554	2555	2556	2557	2558	2559	2560	2561	2562	2563	2564	2565	2566	2567	2568	2569	2570	2571	2572	2573	2574	2575	2576	2577	2578	2579	2580	2581	2582	2583	2584	2585	2586	2587	2588	2589	2590	2591	2592	2593	2594	2595	2596	2597	2598	2599	2600	2601	2602	2603	2604	2605	2606	2607	2608	2609	2610	2611	2612	2613	2614	2615	2616	2617	2618	2619	2620	2621	2622	2623	2624	2625	2626	2627	2628	2629	2630	2631	2632	2633	2634	2635	2636	2637	2638	2639	2640	2641	2642	2643	2644	2645	2646	2647	2648	2649	2650	2651	2652	2653	2654	2655	2656	2657	2658	2659	2660	2661	2662	2663	2664	2665	2666	2667	2668	2669	2670	2671	2672	2673	2674	2675	2676	2677	2678	2679	2680	2681	2682	2683	2684	2685	2686	2687	2688	2689	2690	2691	2692	2693	2694	2695	2696	2697	2698	2699	2700	2701	2702	2703	2704	2705	2706	2707	2708	2709	2710	2711	2712	2713	2714	2715	2716	2717	2718	2719	2720	2721	2722	2723	2724	2725	2726	2727	2728	2729	2730	2731	2732	2733	2734	2735	2736	2737	2738	2739	2740	2741	2742	2743	2744	2745	2746	2747	2748	2749	2750	2751	2752	2753	2754	2755	2756	2757	2758	2759	2760	2761	2762	2763	2764	2765	2766	2767	2768	2769	2770	2771	2772	2773	2774	2775	2776	2777	2778	2779	2780	2781	2782	2783	2784	2785	2786	2787	2788	2789	2790	2791	2792	2793	2794	2795	2796	2797	2798	2799	2800	2801	2802	2803	2804	2805	2806	2807	2808	2809	2810	2811	2812	2813	2814	2815	2816	2817	2818	2819	2820	2821	2822	2823	2824	2825	2826	2827	2828	2829	2830	2831	2832	2833	2834	2835	2836	2837	2838	2839	2840	2841	2842	2843	2844	2845	2846	2847	2848	2849	2850	2851	2852	2853	2854	2855	2856	2857	2858	2859	2860	2861	2862	2863	2864	2865	2866	2867	2868	2869	2870	2871	2872	2873	2874	2875	2876	2877	2878	2879	2880	2881	2882	2883	2884	2885	2886	2887	2888	2889	2890	2891	2892	2893	2894	2895	2896	2897	2898	2899	2900	2901	2902	2903	2904	2905	2906	2907	2908	2909	2910	2911	2912	2913	2914	2915	2916	2917	2918	2919	2920	2921	2922	2923	2924	2925	2926	2927	2928	2929	2930	2931	2932	2933	2934	2935	2936	2937	2938	2939	2940	2941	2942	2943	2944	2945	2946	2947	2948	2949	2950	2951	2952	2953	2954	2955	2956	2957	2958	2959	2960	2961	2962	2963	2964	29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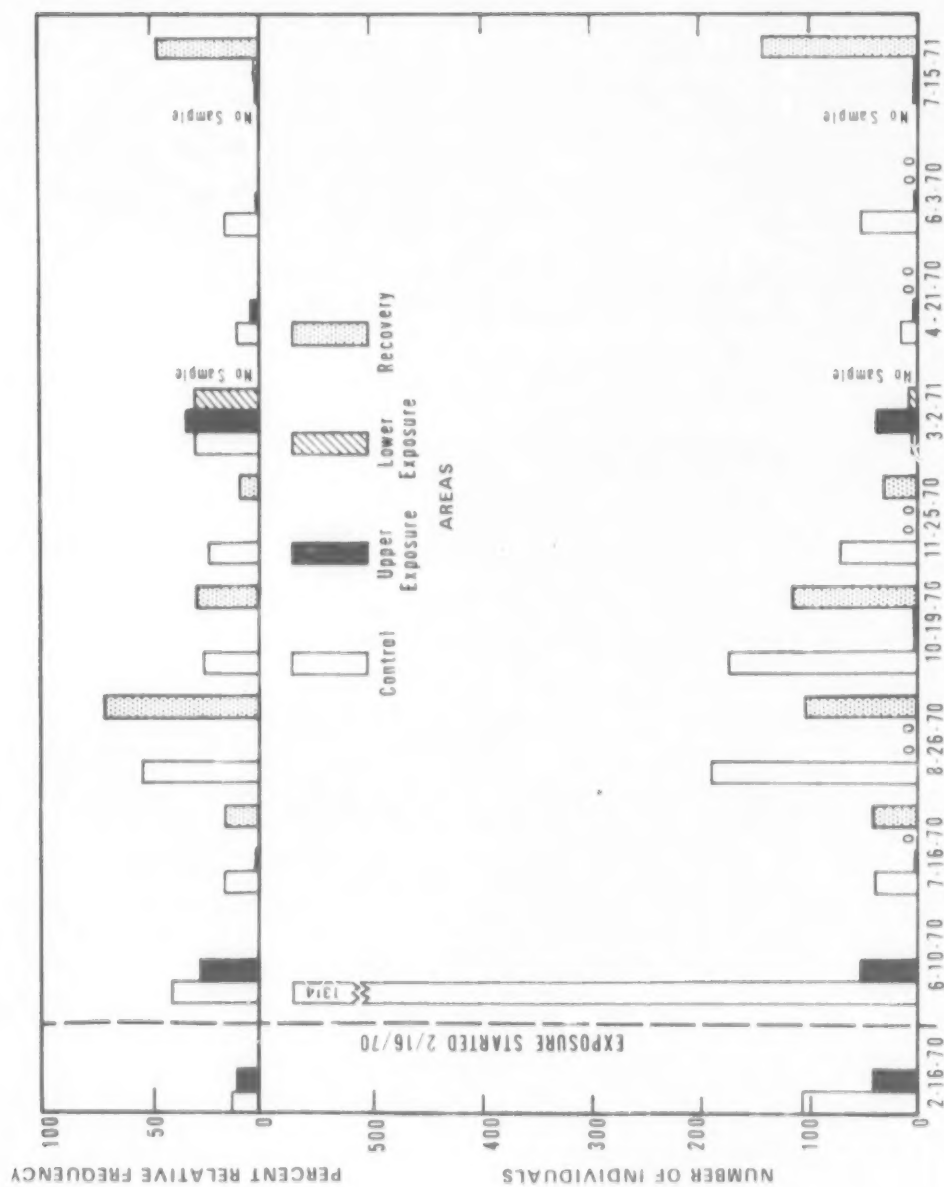


Figure 26. Ephemeroptera (mayflies): natural substrate collections.

concentrations in the exposure area were too high. The average copper concentrations at the upper exposure, lower exposure, and recovery sampling locations for the period June through September 1970 were 114, 58, and 17 $\mu\text{g/L}$, respectively. Mayflies are both terrestrial and aerial as adults and thus have the potential to deposit eggs in all portions of Shayler Run. The relatively few mayflies captured in the exposure area from July 1970 until the end of the study are believed to have been produced in the control area, and the young were probably transported downstream during high water. The reduction in the mayfly population in the exposure area is attributed to copper.

Amphipoda (scuds)

This group of organisms was represented by three species in the stream, Crangonyx anomalus, C. setodactylus, and Synurella dentata. Their normal habitat is shallow, slow-moving water where aquatic vegetation and organic detritus are present. Collections from basket samplers and natural substrates (Tables 15 and 17) indicate that scuds were more abundant during the spring and early summer, and the population levels in the control and exposure areas were similar. Also, there was little reproduction or survival during 1970 and 1971, but the cause is not known.

Results from the weir-screen collections (Table 16, Figure 27) show a considerable difference in the numbers of scuds moving downstream from the exposure area and the numbers from the control area during the first few months of exposure. For the period of February 23 to May 8, 1970, there was a 25-fold difference (3,937-157) between the number of scuds collected on the exposure screens and the number on the control screens. The high drift rate from the exposure area is attributed to the addition of copper.

Chironomidae

The population of Chironomids in the stream was composed of 30 species (Table 13). Peak population levels normally occurred in May, June, and July (Tables 15, 16, and 17, Figures 28 and 29). Like mayflies, the adults are both terrestrial and aerial and may deposit eggs in surface water.

Basket-sampler collections made before exposure to copper showed that the Chironomid population in the exposure area was somewhat greater than that of the control area. The frequency of occurrence (Figure 28) of Chironomids per individual samples averaged less than 10% for the control station and the two exposure-area stations. During the exposure period, however, there was a substantial increase in the Chironomid population of the exposure area compared to that of the control. The average frequency of occurrence per sample increased to 15% for the control, but there was a major increase at the exposure stations to 60% and 43% for upstream and downstream stations, respectively. The percentage increase shows the dominance of Chironomids in the exposure area during the exposure period over some of the more common forms that had been adversely affected by copper, such as isopods and mayflies. It is reasonable to believe that, with the reduction in numbers of other common forms of macroinvertebrates and the reduction in the exposed fish population, Chironomids that could withstand the copper concentration had less competition for food and space along with less predation and therefore, flourished during this period.

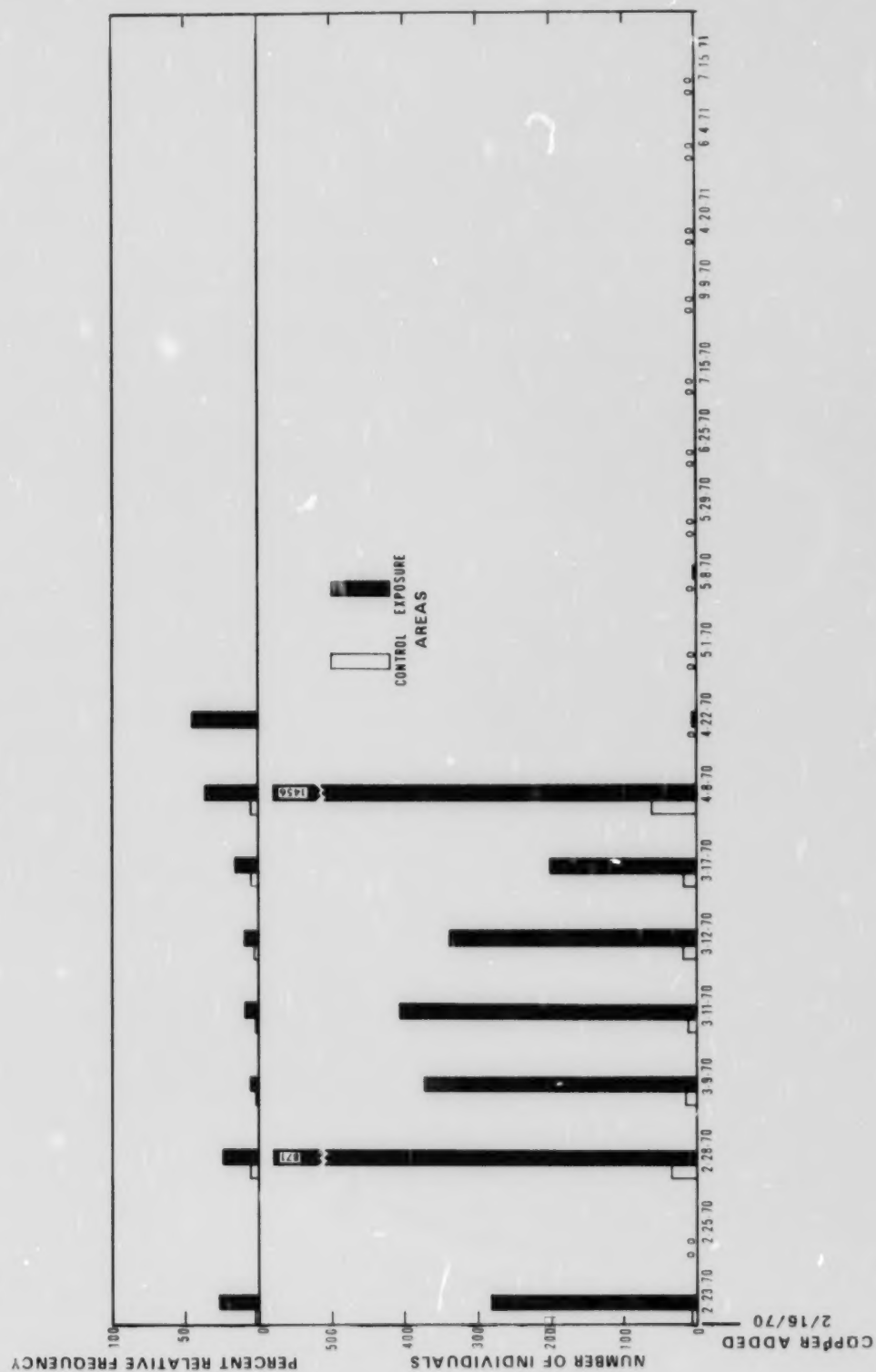


Figure 27. Amphipoda (scuds): weir-screen collections.

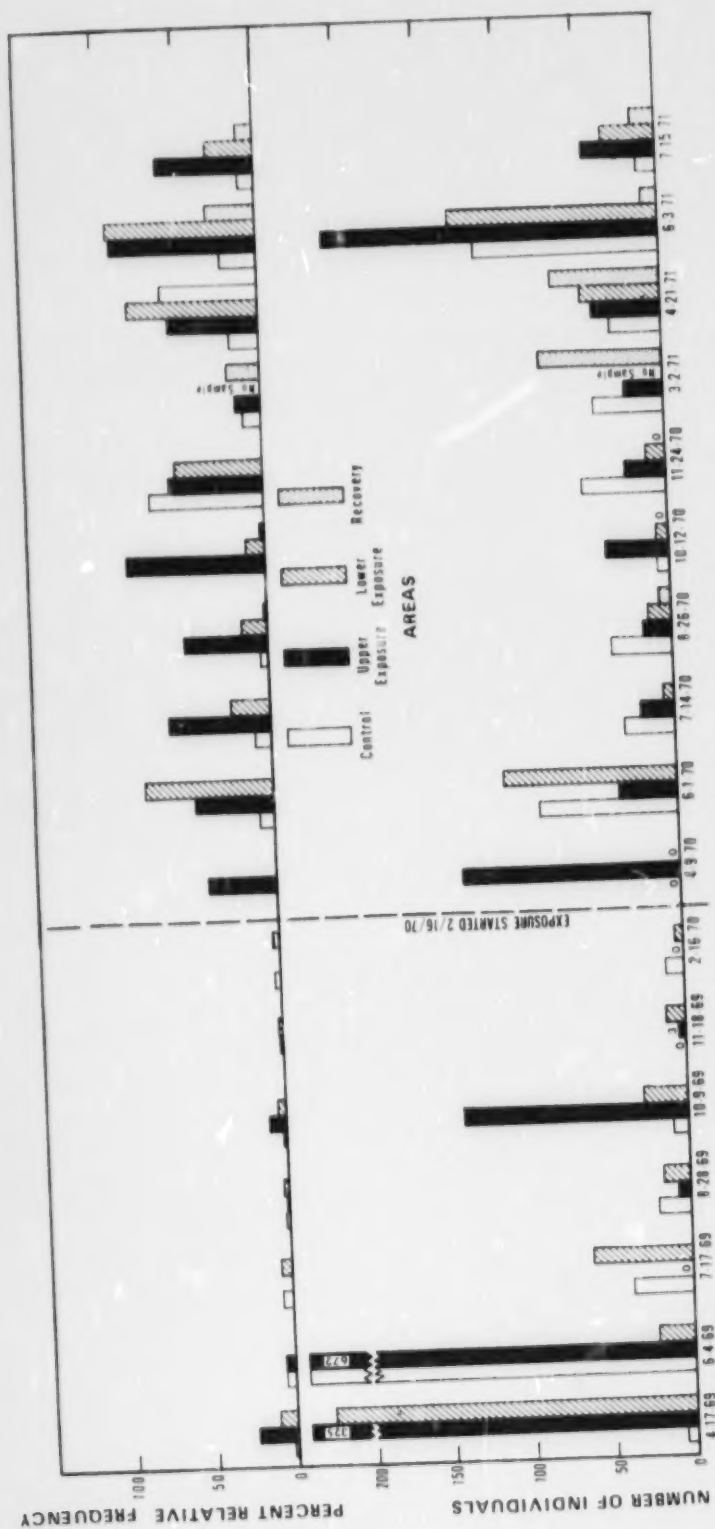


Figure 28. Chironomidae (Chironomids): basket-sampler collections.

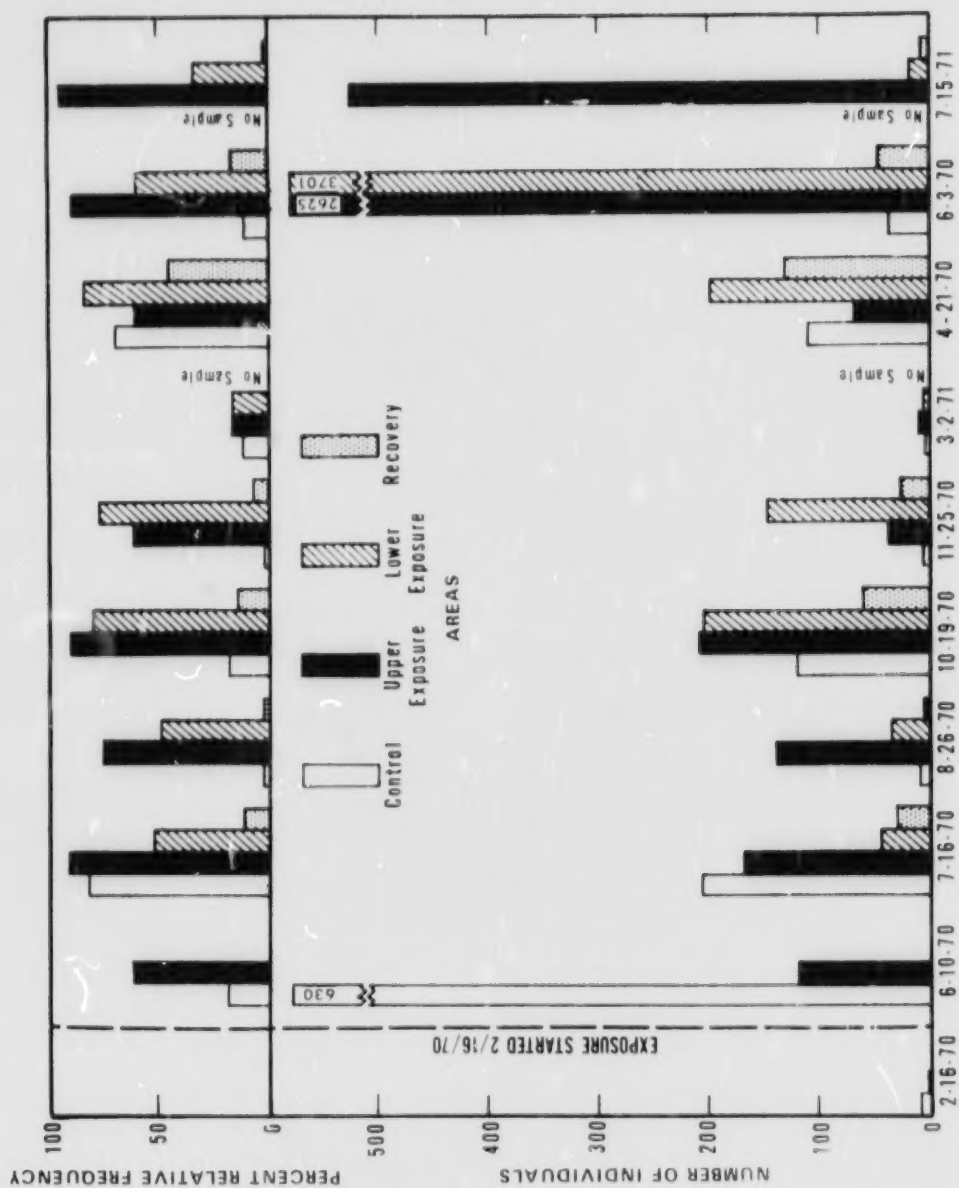


Figure 29. Chironomidae (Chironomids): natural substrate collections.

Psephenidae (riffle beetles)

Only one species of riffle beetle, Psephenus herricki, was present in the collections, and it was common during the pre-exposure period in the control area and in even greater numbers in the exposure area (Tables 16 and 17, Figures 30 and 31). Riffle beetle larvae adhere very tightly to rocks in shallow, swift-moving waters, and thus they contributed little to the number of drift organisms collected on the weir screens (Table 16). The adults are semi-aquatic and can fly. Eggs, therefore, could be deposited in any area of the stream regardless of copper concentration.

During the exposure period, from June 1970 onward, the population of riffle beetles was reduced in the exposure area as compared to the population during the pre-exposure period (Table 15, Figure 30). The population in the control area during the exposure period was comparable to the pre-exposure level. The recovery area station had a population during the exposure period greater than that at the exposure stations and somewhat similar to that at the control station. This response was similar to that of mayflies, in that the population of riffle beetles was greater in the recovery area where the copper concentration was lower. It appears that copper adversely affected the riffle beetle population in the exposure area.

Trichoptera (caddisflies)

Few caddisflies were collected in basket samplers as compared with natural substrate sampling. The results from rock scrubbing were similar to those of Anderson and Mason (1968) when they made comparisons of sampling success by Ekman dredge and basket sampler. The data indicate that caddisflies were not greatly affected by copper (Figure 32, Tables 15, 16, and 17).

Periphyton

Of the various parameters examined, species composition was found to be the most sensitive and informative measure of the effects of copper on the periphyton community. Two of the dominant species of algae were eliminated from the periphyton: the diatom Cocconeis placentula var. euglypta, and the filamentous green alga, Cladophora glomerata. Cocconeis placentula, which commonly contributed 85-98% of the summer diatoms in the exposure area before the addition of copper, was replaced by three species of diatoms--Nitzschia palea, Navicula minima, and N. seminulum var. hustedtii. Other species of algae that were more abundant in the treated area than in the control area of the stream were the filamentous blue-green alga Schizothrix calcicola and the desmids Cosmarium granatum and C. subprotumidum.

A manuscript presenting more detailed information concerning effects of copper on the Shayler Run periphyton community is being prepared by C. I. Weber and B. J. McFarland of the Environmental Monitoring and Support Laboratory, U. S. Environmental Protection Agency, Cincinnati, Ohio.

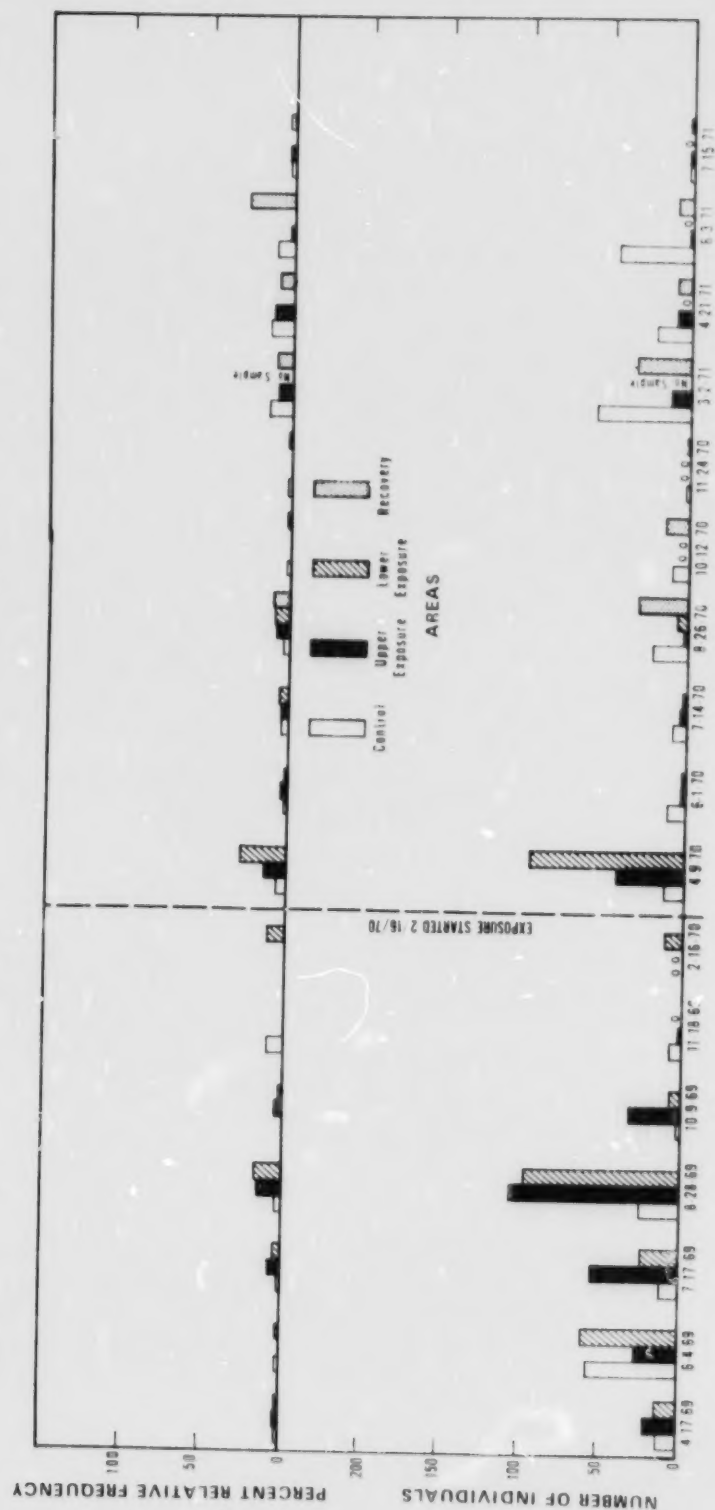


Figure 30. Psephenidae (riffle beetles): basket-sampler collections.

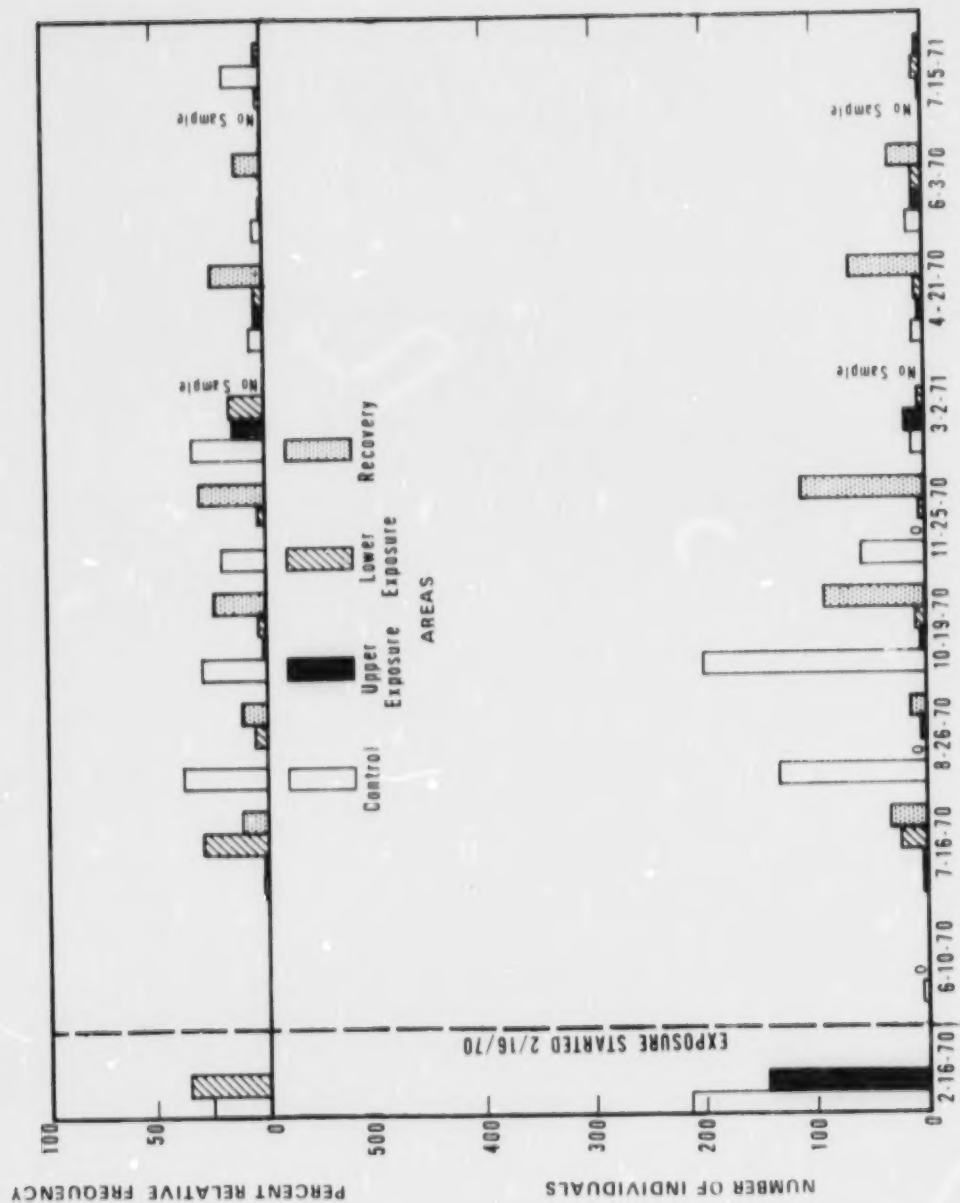


Figure 31. Psephenidae (riffle beetles): natural substrate collections.

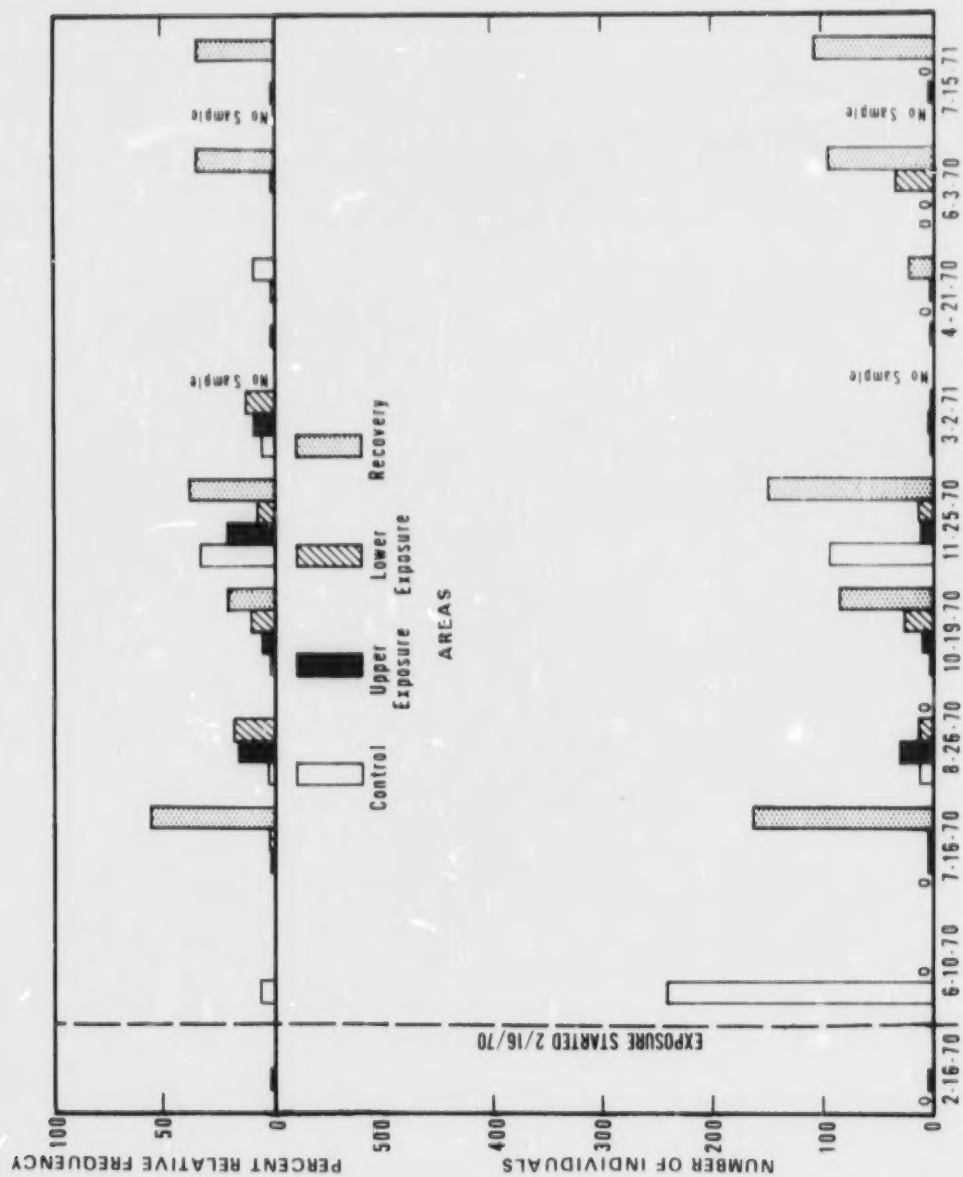


Figure 32. Trichoptera (caddisflies): natural substrate collections.

SECTION XII

FISH-STOMACH ANALYSIS

INTRODUCTION

Continuous observations of feeding habits reveal that selectivity of organisms by fishes, even within the limits of a single ration and absolutely stable conditions, is not a constant value, and any quantitative measurement is a function of the food consumed (Ivelev, 1961). It was assumed that if macroinvertebrates were reduced or eliminated by copper, the number of fish dependent upon this food source in the exposure area would be reduced by migration, starvation, and predation. If fish were to survive, a shift in diet to other food materials would have to occur. The phenomena of electivity of feeding based upon a preference shown by the stream fishes and upon the degree of accessibility of food organisms could not be assessed under natural conditions. Therefore the predominant organisms found in fish stomachs were compared with data obtained from the artificial and natural substrates used during the study. This is in keeping with the proven results that show that electivity values in any biological system are the ratio between the concentrations of the ingredients making up the food complex.

It was thought originally that five or six fish species would lend themselves to stomach-content analysis, but this was not the case. To be useful the species would have to be common in both the control and exposure areas throughout the study period, and their stomach contents would have to be identifiable. The two species that met these requirements were the green sunfish and the orangethroat darter, both of which spawned rather well in the exposure area.

METHODS

Fish specimens analyzed for stomach content were from the eight biannual fish collections discussed earlier. Ten specimens each of green sunfish and orangethroat darters were chosen from both control and exposure area collections. Fish were selected so that the lengths of individual specimens within each group from control and exposure collections were duplicated as close as possible. The orangethroat darters were analyzed for all eight biannual fish collections, four pre-exposure collections and four exposure-period collections. Green sunfish stomach contents, however, were only analyzed for the two 1969 pre-exposure collections and the following four exposure-period collections. More than 85% of the stomach contents from both species could be identified for all specimens. Since all fish of 60-mm total length or greater were injected with AFA after collecting, preservation of the stomach contents was excellent.

Total lengths of each fish were recorded, and their stomachs were removed intact. Stomach contents were removed with fine forceps and placed in a petri dish containing distilled water. The materials were examined with the aid of a

stereomicroscope or a compound microscope when necessary at magnifications from 10X to 1000X. Organisms found in the stomach materials were identified and enumerated.

RESULTS AND DISCUSSION

The organisms found in the stomachs of orangethroat darters and green sunfish are listed in Tables 18 and 19, respectively. The numbers shown for each group of macroinvertebrates are the totals from 10 stomachs. The group classified as other aquatic macroinvertebrates consists of the following: crane fly, stonefly, leech, and nematode. Based on their occurrence, fish and terrestrial invertebrates were not of major importance in the diet of orangethroat darters during either the pre-exposure or the exposure period.

Orangethroat Darter

At least 9 of the 10 stomachs per sample contained food, including the samples from the exposure area during the exposure period. In general, more organisms were present in orangethroat darter stomachs in the exposure-period collections than in the pre-exposure collections for both the control and exposure samples (Table 8, Figure 33). Based on pre-exposure data, the dominant food items for this species of fish were sowbugs, mayflies, copepods, and Chironomids. This would be expected since sowbugs, mayflies, and Chironomids were the most common benthic organisms in the stream during the pre-exposure period.

During the exposure period sowbugs and mayflies were practically eliminated in the exposure area, and this, in turn, was reflected in the stomach contents of the orangethroat darters collected in the fall of 1970 and in both spring and fall of 1971 (Table 18, Figure 34 and 35). The sowbugs present in the stomachs from the fish in the exposure area for this period were found only in the spring collections, and these specimens were small early instars that could have passed through the weir screens. The mayflies, which were present in the orangethroat darter stomachs from the exposure area for the May 15, 1970, collection, were probably produced in the exposure area.

The mayfly population in the exposure area was still fair into June 1970. In the next three collections only two mayfly specimens were found in orangethroat darter stomachs from the April 7, 1971, collection, and these may have been washed from the control area during high water.

During the spring months copepods contributed large numbers to the diet of orangethroat darters (Table 18, Figure 36), except for the 1969 spring collection which was made earlier than the other spring collections when the normal spring population increase of copepods had not yet occurred. The water temperature was still low (3° C), and the fish probably were not feeding heavily. During the exposure periods copepods made up a higher percentage of the spring diet of the orangethroat darter in both the control and exposure area.

The effects of the copper exposure on copepods in the stream are not known. Copepods were not normally captured or studied during the project, and therefore population comparisons were not possible. Because of their small size, many of the specimens found in the stomachs may have originated in the control area and passed through the weir screens.

TABLE 18. NUMBER OF ORGANISMS IN THE STOMACHS OF ORANCETHROAT DASTERS, SHAYLER RUN, 1968-71

Collection area	Pre-exposure period				Exposure period			
	Spring 4/11/68		Fall 9/26/68		Spring 3/18/69		Fall 9/26/69	
	Control	Exposure	Control	Exposure	Control	Exposure	Control	Exposure
Aquatic macroinvertebrates								
Diptera (true flies)	78	44	41	54	14	9	46	7
Chironomidae (midges)		1	1		1			
Simuliidae (blackflies)								
Coleoptera (beetles)	1	2		1				2
Psephenidae (water penny)		1	1					
Elmidae (riffle beetles)	2	1	32	18	1	1	28	11
Ephemeroptera (mayflies)	2			3	1	2	1	
Trichoptera (caddisflies)	5	5			2	3		2
Amphipoda (scuds)	70	13	14	6	4	3		
Copepoda (Copepoda)								
Decapoda (crayfish)	37	12	22	5	3	1	31	18
Isopoda (sowbug)	7	21	25	51	3	3	1	
Ostracoda (shrimps)	3	9						
Oligochaeta (worms)	1					1		
Other aquatic macroinvertebrates							1	2
Pisces (fish)								
Terrestrial invertebrates								
Total	206	108	136	139	31	21	108	46
Number of empty stomachs	1	0	0	0	1	1	0	1
Length of fish-range mm	45-56	45-57	45-56	45-58	44-52	46-55	48-58	49-59

TABLE 19. NUMBER OF ORGANISMS IN THE STOMACHS OF GREEN SUNFISH, SHAYLER RUN, 1969-71

Collection Area	Pre-exposure period			Exposure period					
	Spring		Fall	Spring		Fall		Spring	Fall
	3/18/69	9/21/69		5/15/70	10/6/70	5/7/71	10/5/71		
Aquatic macroinvertebrates	Control	Exposure	Control	Exposure	Control	Exposure	Control	Exposure	Control
	6	3	2	9	4	2	2	27	2
	4			1					
	1	2	14	3	3	10	17	2	17
	9	23	6	4	11	11	14	2	14
	15			3	3	38	9	9	1
				2	2	21	1	4	1
				4	3	1	1	2	1
	3	59	22	48	90	34	20	2	1
	4	6	1	5	1	1			
Pisces (fish)	1	1	4	2	9	6	1	5	5
Terrestrial invertebrates									
Total	51	28	62	100	127	131	54	50	12
Number of empty stomachs	0	1	2	1	2	3	4	1	4
Length of fish-range mm	103-152	92-143	101-163	93-171	55-105	47-98	101-164	101-173	80-120

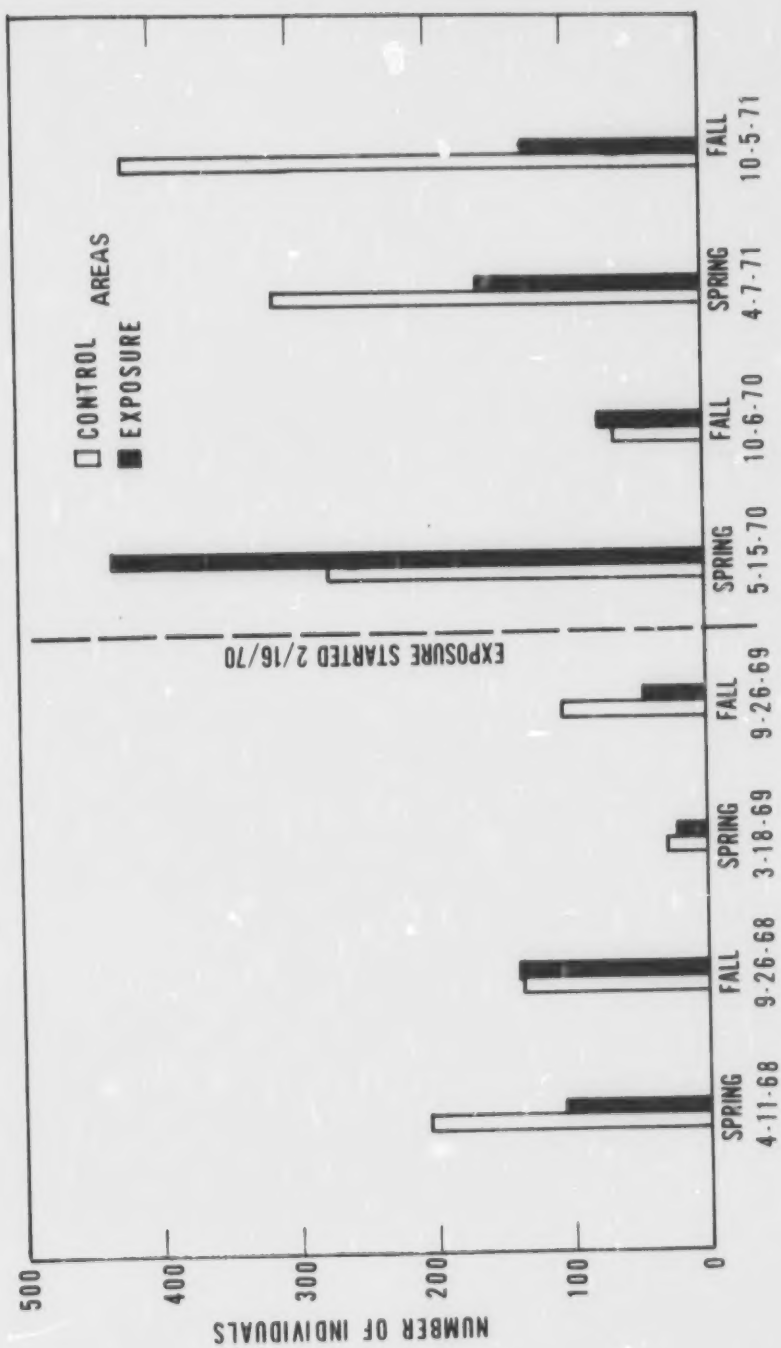


Figure 33. Total numbers of macroinvertebrates found in orangethroat darter stomachs.

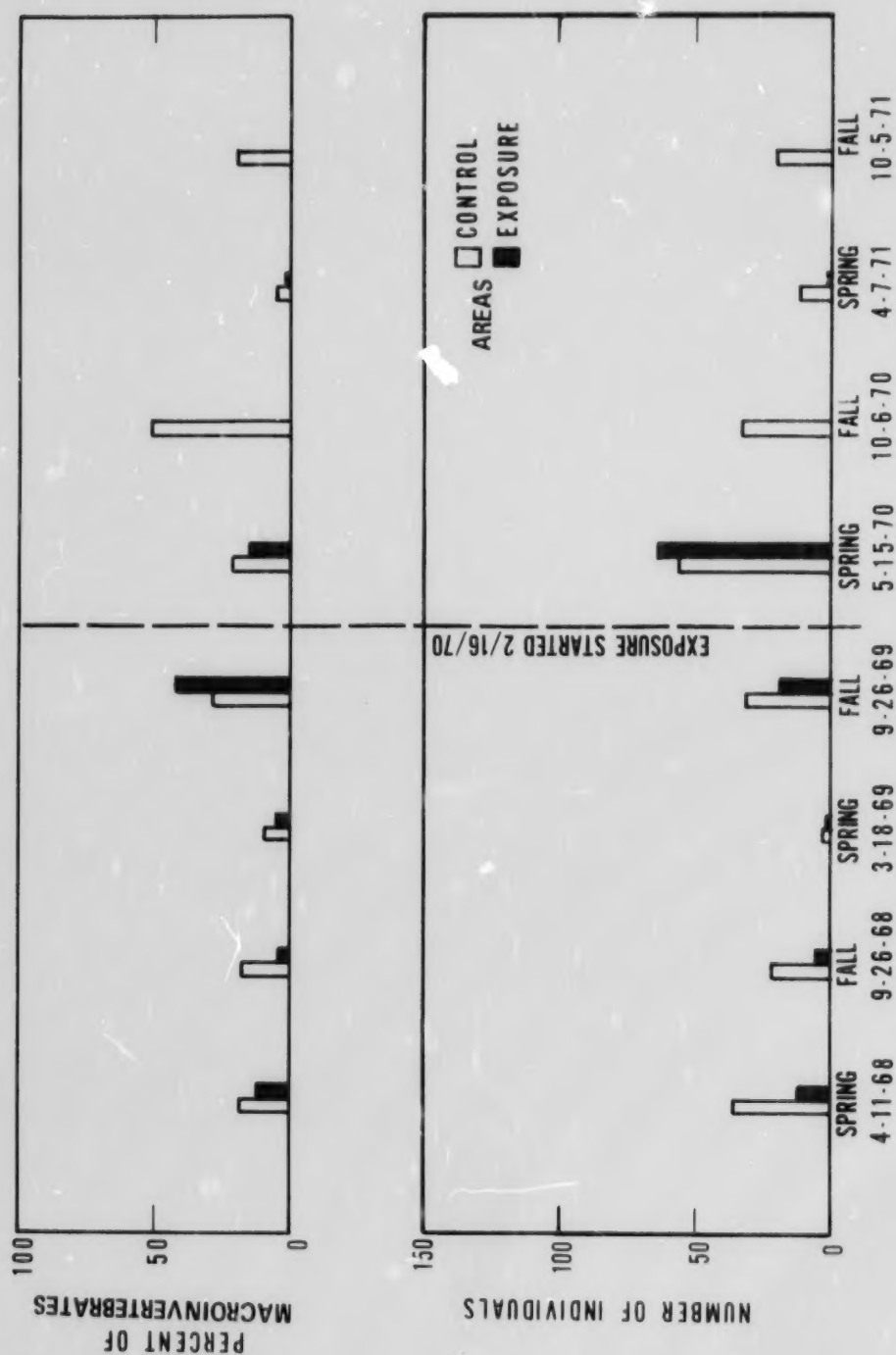


Figure 34. Isopoda (sowbugs) found in orangethroat darter stomachs.

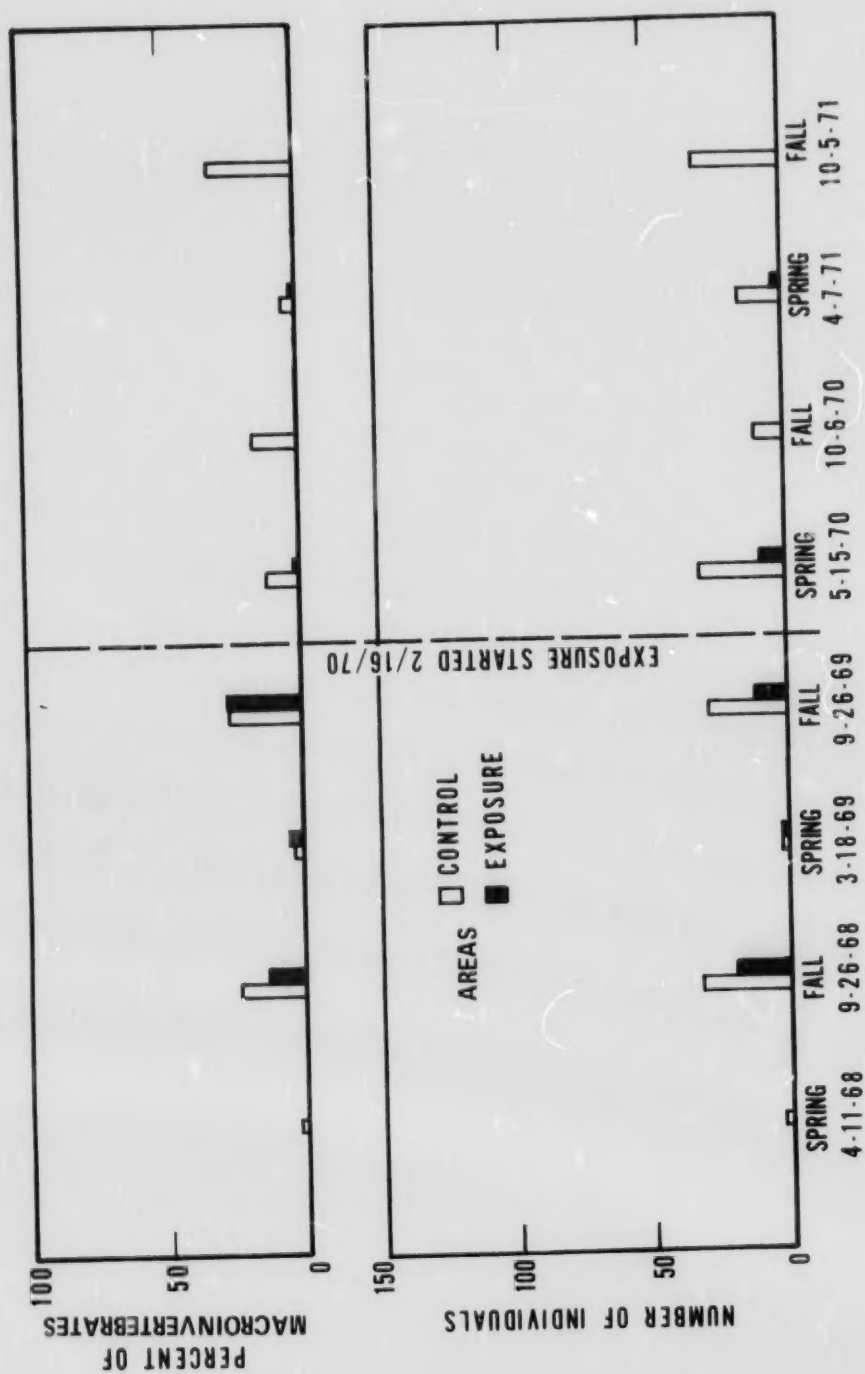


Figure 35. Ephemeroptera (mayflies) found in orangethroat darter stomachs.

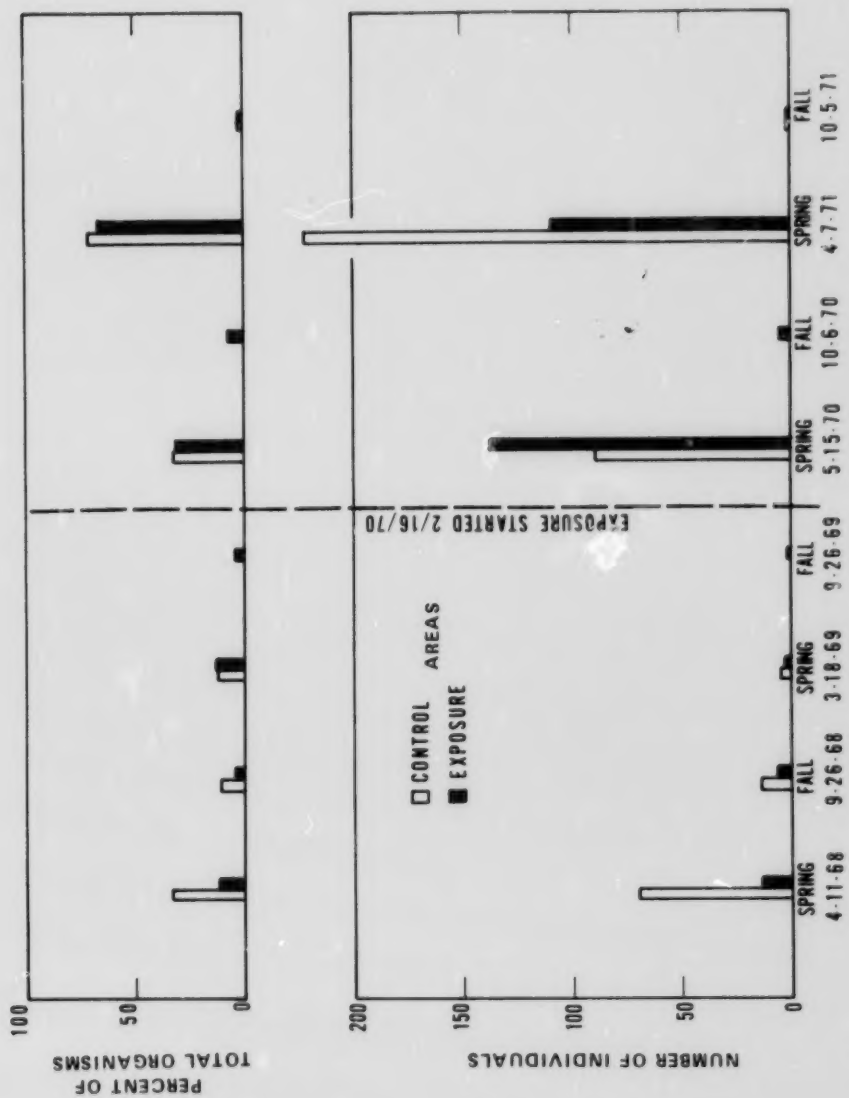


Figure 36. Copepoda found in orangethroat darter stomachs.

The chironomids as a group provided the greatest numbers of individuals in the diet of the orangethroat darter in both the control and exposure areas for the pre-exposure and exposure periods (Table 18, Figure 37). In general, chironomids were not adversely affected by the addition of copper to the stream, and the stomach analyses indicate that they composed a larger portion of the diet of the orangethroat darter in the exposure area during the exposure periods.

Green Sunfish

The results of the green sunfish stomach analysis are presented in Table 19. The data were treated as they were for the orangethroat darter. The group classified as other aquatic macroinvertebrates consists of the following: Stratiomyidae, Elmidae, Plecoptera, Megaloptera, Hirudinea, Ostracoda, Physa, and salamander larvae. All of these were found in stomachs of green sunfish from the control area only, except the salamander larvae of which only one specimen was observed from the stomach of a green sunfish from the exposure area.

A few empty stomachs were found during the pre-exposure-period collections. A greater number of empty stomachs occurred during the exposure period, but the numbers were similar for both the control and exposure specimens. No relationship between empty stomachs and exposure to copper was found.

The numbers of organisms present in green sunfish stomachs in the pre-exposure collections were similar for control and exposure-area specimens (Table 19, Figure 38). However, the numbers of organisms present in green sunfish stomachs were from two to five times greater in control specimens than in exposure specimens in collections during the exposure period.

During the pre-exposure period five groups of food organisms were present in the control fish stomachs at levels greater than 10% of the total numbers. These were Chironomids, sowbugs, mayflies, scuds, and riffle beetles. During the same period, however, only mayflies, scuds, and sowbugs were above the 10% level in the stomachs of exposure-area fish.

The reduced population of sowbugs and mayflies in the exposure area is reflected by the stomach contents of exposure-area green sunfish for the same period. In the first exposure-period collection, 19 sowbugs were found in fish stomachs from the exposure area. For the remaining three collections, only one or two were found per collection (Figure 39). During the pre-exposure period mayflies made up approximately 20% of the food organisms in the diet in both control and exposure areas. During the exposure period this level was maintained for the control area (Figure 40), but only two specimens of mayflies were present in green sunfish stomachs from the exposure-period collections. This supports the observations during the macroinvertebrate study.

Scuds normally occurred in stomachs of fish collected in the spring, but not in those collected during the fall. Scuds found in the fish stomachs during the pre-exposure spring collection were all adults, since reproduction of this form had not yet occurred. Specimens of scuds from green sunfish stomachs during the exposure period were of mixed sizes in control-area fish stomachs, but were all small (early instars) in the stomachs from exposure-area fish. Adult scuds were either not present or at least were very scarce in the exposure area. It is also possible that the newly hatched young present in the exposure-area fish stomachs were not produced in the exposure area, but were from the control area.

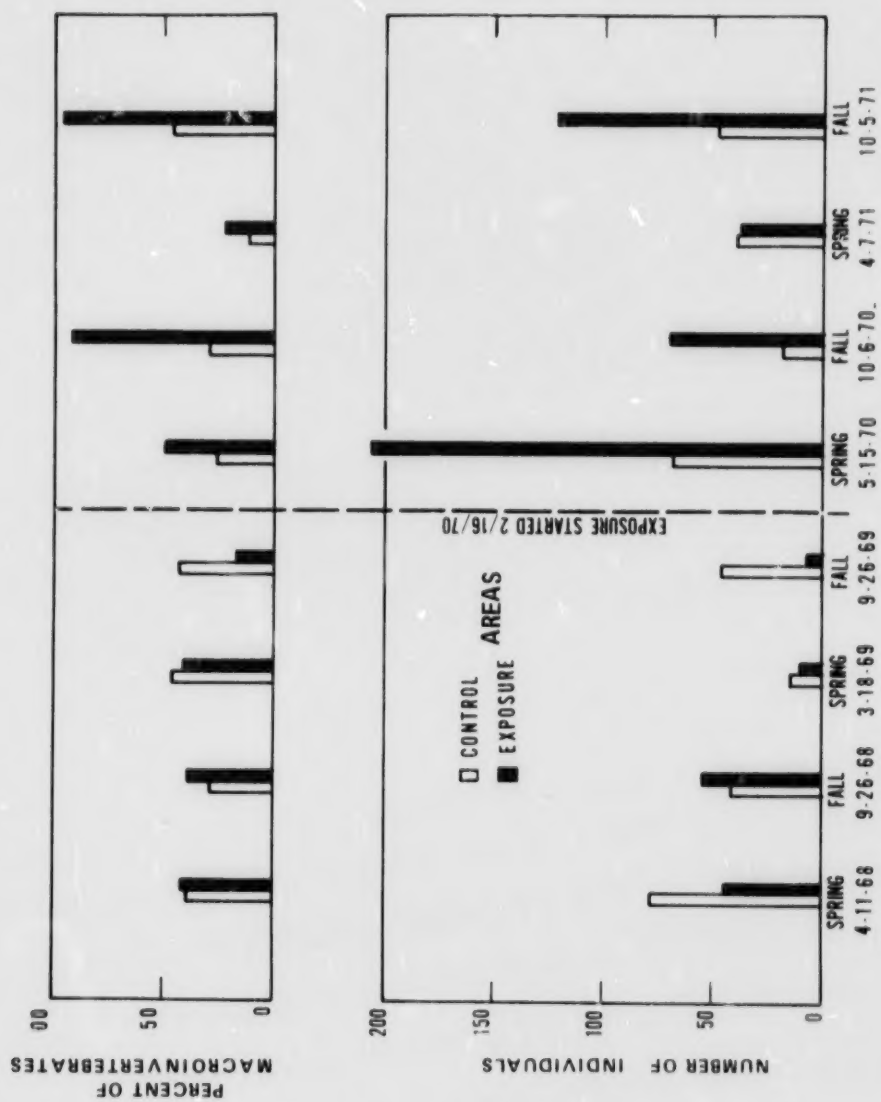


Figure 37. Chironomidae (Chironomids) found in orangethroat darter stomachs.

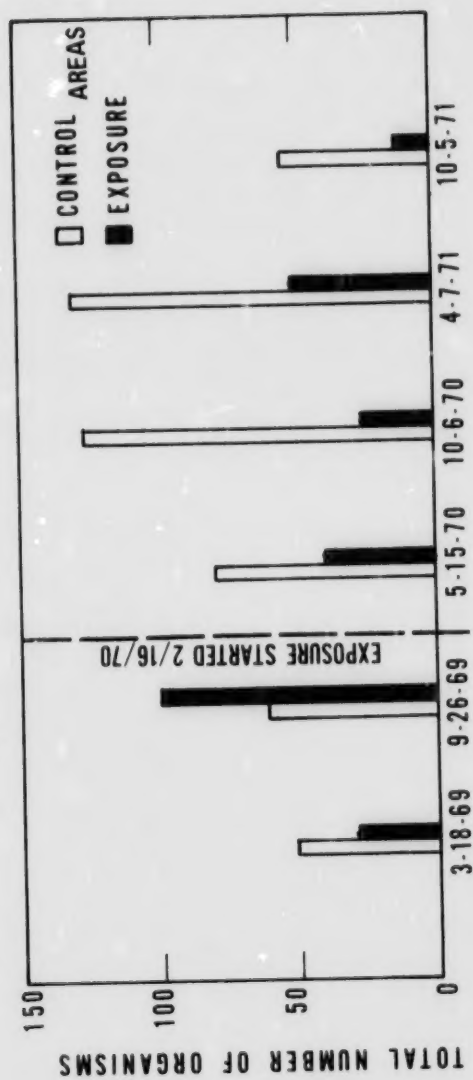


Figure 38. Total numbers of organisms found in green sunfish stomachs.

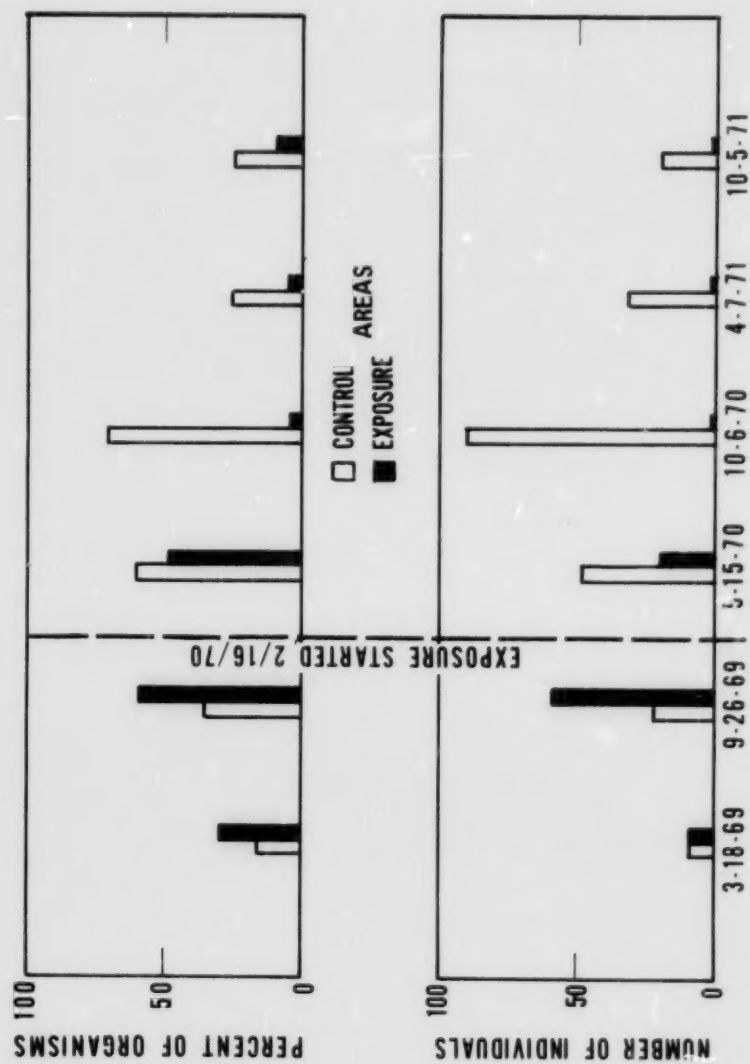


Figure 39. Isopoda (sowbugs) found in green sunfish stomachs.

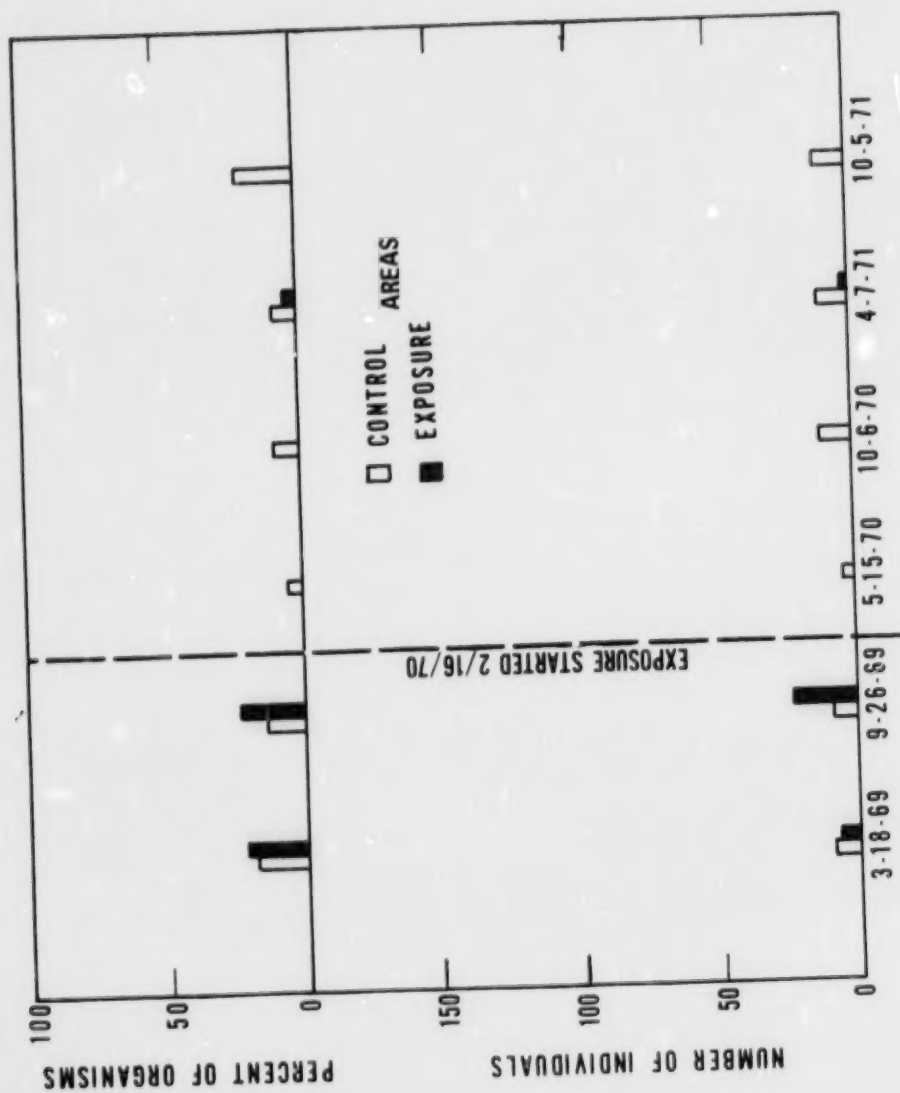


Figure 40. Ephemeroptera (mayflies) found in green sunfish stomachs.

Chironomids, even though present in both control and exposure areas throughout the study period, were not as important in the green sunfish diet as they were in the diet of the orangethroat darter (Tables 18 and 19, Figure 41). In one collection during the exposure period, however, Chironomids composed as much as 54% of the total organisms present in stomach contents of green sunfish in the exposure area.

Fish composed a portion of the diet of green sunfish during the study. Even though few were actually present in stomachs, a number of these specimens were large when compared to other food forms and thus did constitute a considerable volume of the diet (Table 19, Figure 42). The number of fish present in the stomachs was similar in control-area and exposure-area sunfish for both the pre-exposure and exposure periods. If common food organisms such as sowbugs, mayflies, and scuds were reduced, a shift in diet to fish might occur. Such a shift would be mitigated because there was also a major reduction in the population of fishes in the exposure area during the same period.

Terrestrial organisms were not common in the stomachs of green sunfish during the pre-exposure period (Table 19, Figure 43). During the exposure period, however, a greater number were present in the stomach contents, especially for the exposure-area specimens. This is not unexpected since common aquatic food organisms of green sunfish had been reduced. Even though only a limited shift in food type did occur in the exposed green sunfish population, if copper were added to the stream for a longer period, food could become the limiting factor for the population of green sunfish in the exposure area.

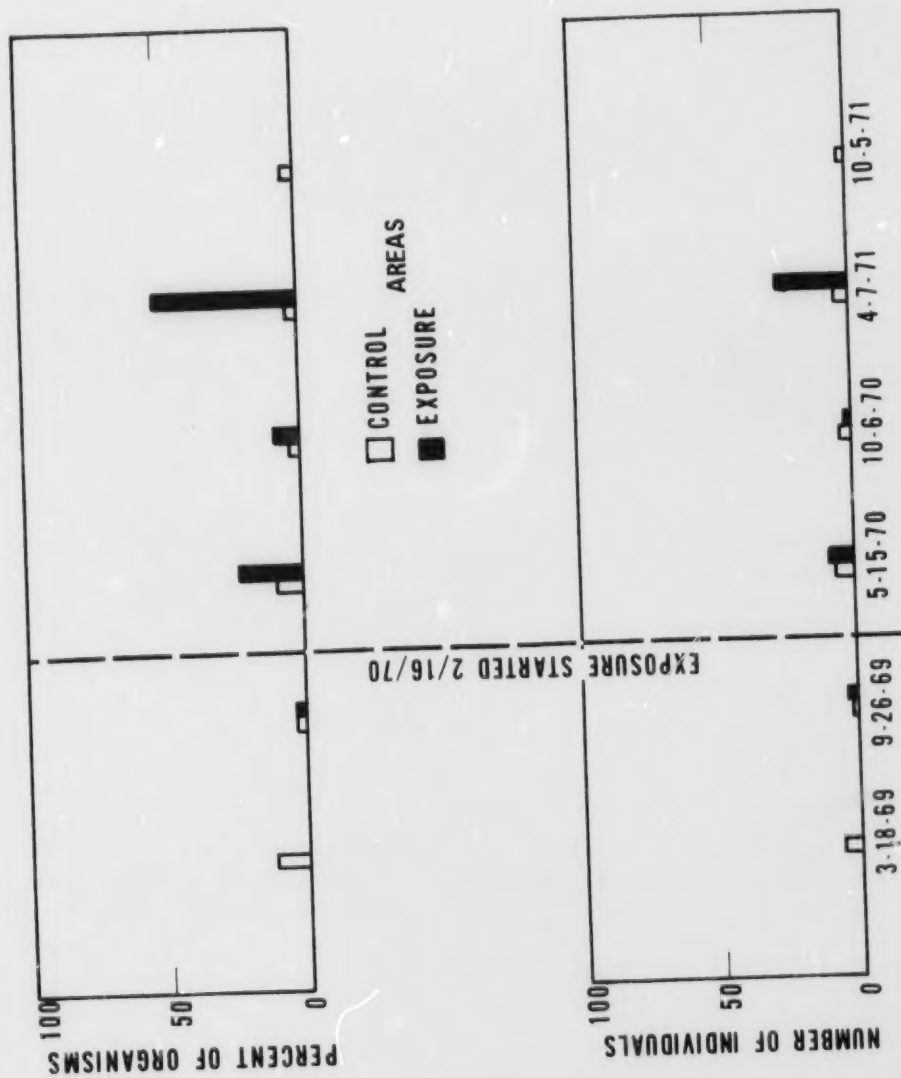


Figure 41. Chironomidae (Chironomids) found in green sunfish stomachs.

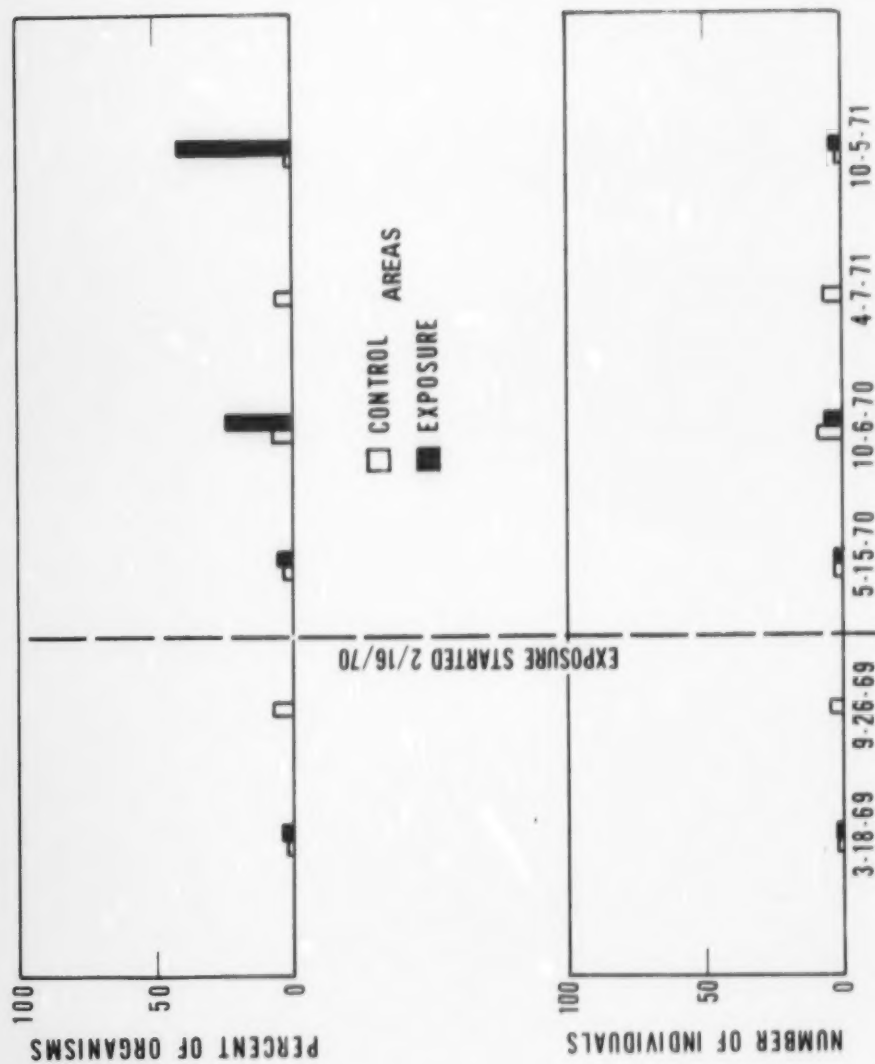


Figure 42. Fish found in green sunfish stomachs.

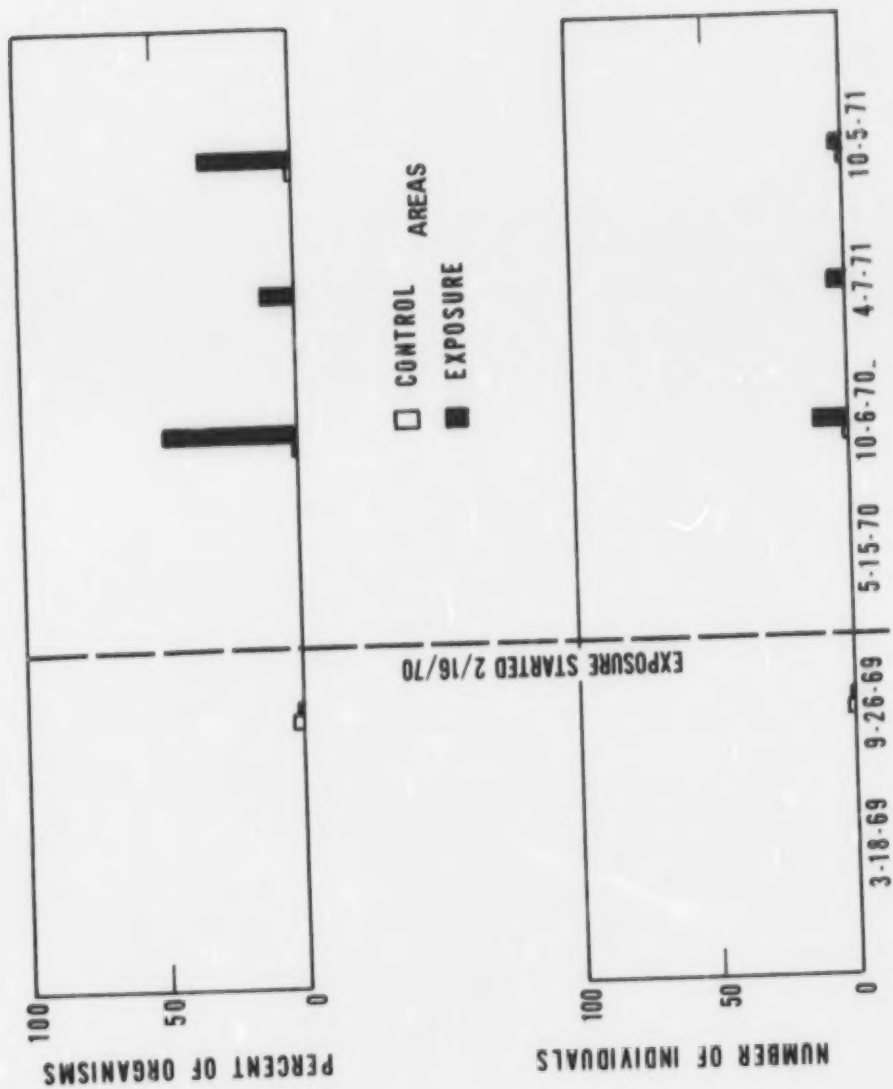


Figure 43. Terrestrial organisms found in green sunfish stomachs.

PART C -- LABORATORY STUDIES

SECTION XIII

ACUTE STUDIES

INTRODUCTION

In preparation for long-term exposure of the stream to a constant concentration of copper, laboratory toxicity tests were conducted to evaluate acute copper toxicity to fish in Shayler Run water. The data from these toxicity tests were to be used not only for the evaluation of acute toxicity, but also for predicting "safe" and "unsafe" concentrations in Shayler Run. To evaluate acute copper toxicity in Shayler Run water, two major objectives were pursued: (1) to determine the effect of changes in water quality of Shayler Run on acute toxicity, and (2) to determine the relative sensitivity of several common stream species.

Because many water quality factors influence copper toxicity, it was necessary to know how acute toxicity of copper in Shayler Run water varied with the varying water quality of the stream. Most of these toxicity tests were conducted with unmodified Shayler Run water. A few were made, however, with water from the Newtown Fish Toxicology Station (NFTS) laboratory (standard water) and with modified Shayler Run water. The bluntnose minnow, a common Shayler Run fish species, and the fathead minnow were used in these toxicity tests. Static tests were used so that more bioassays could be conducted and so that large volumes of water would not be needed.

To determine the copper concentration to be used in the stream exposure, it was desirable to know what concentrations of copper in Shayler Run water might be acutely lethal to the more sensitive species. Also, the acute toxicity data were to be used for predicting direct chronic toxicity of copper in the Shayler Run exposure to species with which streamside chronic tests could not be conducted. This was to be done by assuming that the order of sensitivity of the stream species to copper would be the same in both short-term and long-term exposures.

The model for predicting long-term direct toxicity to the species in the stream exposure was the laboratory chronic toxicity studies of Mount (1968) and Mount and Stephan (1969). They proposed that the application factor for copper be calculated by dividing the maximum acceptable toxicant concentration (MATC) of a chronic test by the LC50 concentration of an acute test, using the same species of fish in the same dilution water. They suggested that the application factor might be applicable to other species of fish. The application factor assumes that the relative sensitivity of various species in terms of the MATC's should be the same as the relative sensitivities of the species in terms of the LC50 in the same water. The application factor does not assume that the relative sensitivities of different species must be the same in all waters.

The relative sensitivity studies were made with flow-through testing to more closely duplicate stream conditions. Sensitivity of fish species was determined and evaluated in terms of the 96-hr LC50 values of copper. These species were tested at different times in the standard water, and a series of three tests was conducted with different species at the same time in Shayler Run water.

METHODS

Water Quality Studies

These static acute mortality tests were conducted according to routine bioassay methods recommended by the American Public Health Association (1965). The physical and chemical characteristics of Shayler Run water and copper concentrations were measured as reported in Part A (Water Quality-Chemical). Copper was added to the test chamber from a stock solution of reagent-grade $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ dissolved in distilled water. At least five concentrations with a 0.5 dilution factor were tested; 10 fish were used for each concentration. The experimental design of these bioassays gave estimated LC50 values by using graphical interpolation. In addition, some of the LC50 values were calculated by probit analysis using a computer program based on Chapter 18 "Assays Based on Quantal Response" in "Statistical Methods in Biological Assay" (Finney, 1971).

The bluntnose minnow was used as the test species for the toxicity tests conducted at the NFTS, because it was the most abundant species in Shayler Run and is adaptable to laboratory conditions. All test fish were young-of-the-year individuals collected from Shayler Run. The test fish were held in Shayler Run water at the natural stream temperature, and the fish along with the stream water were brought to the laboratory on the day of testing. Initially the fish averaged 25 mm in length; at the end of the testing period they averaged 50 mm. For a given bioassay the size of the largest fish was not more than 1.5 times the length of the smallest. Initially, 10 fish were tested in 2 L. of solution. As the season progressed and the fish grew, the volume of the test solution was increased so that the weight of fish in the test containers did not exceed 1 g/L. and dissolved oxygen concentrations were maintained above 4 mg/L. The volume for 10 fish was increased to 3, 6, and 10 L. The test fish were added to the 3.8-L. or 19-L. wide-mouth glass jars immediately after the preparation of the test solutions. Tests lasted 48 hr, even though most of the deaths occurred in 24 hr at the test temperature of $24 \pm 2^\circ \text{C}$.

The usual source of Shayler Run water was from just above the V-notch weir, the point of copper introduction. A few bioassays were conducted with Shayler Run water upstream from the entrance of the effluent from the sewage treatment plant and with standard laboratory water. The majority of the bioassays were conducted in unmodified stream water; however, to investigate sources of variation of copper toxicity, the stream water was modified for some tests.

For the tests conducted on site, the fathead minnow was used. The fish were obtained from the Newtown Fish Farm, Ohio Department of Natural Resources, Division of Wildlife, and were acclimated for at least 2 weeks in stream water. These fish ranged from 20 to 71 mm total length, but for an individual test the difference in length between the largest and the smallest fish was less than two times. The test chambers were 19-L. wide-mouth jars and contained 10 L. of

unaerated stream water. The chambers were placed in a flow-through water bath supplied with stream water, and the bioassay was run at ambient stream water temperature. Five fish were randomly assigned to duplicate chambers to give a total of 10 fish per concentration. Numbers of dead fish were recorded every 24 hr for 7 days. Dissolved oxygen, pH, alkalinity, and hardness were measured at the beginning of each test before fish were added and at the end. One hour after copper was added, samples for dissolved copper analysis were taken from one of each set of duplicate test chambers. At the end of the static tests the test solutions were acidified, and samples were taken for total copper analysis.

Relative Sensitivity Studies

Standard Water--

Two flow-through exposure systems were used to test the acute toxicity of copper in the standard water. A proportional diluter (Mount and Brungs, 1967) was used to deliver a control and five toxicant concentrations with a dilution factor of 0.6. The glass test chambers were 30 by 30 by 30 cm high and were calibrated by means of a standpipe to contain 15 L of test solution. The water from the diluter was divided to deliver 500 ml to each duplicate tank. A chemical-metering device was used to deliver reagent-grade copper sulfate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) solution from a Mariotte bottle. The standard dilution water was a mixture of spring water and carbon-filtered demineralized Cincinnati tap water maintained at a hardness of 200 mg/L (as CaCO_3). During the tests the characteristics of this water were as follows: hardness, 196-205 mg/L (as CaCO_3); alkalinity, 148-161 mg/L (as CaCO_3); pH, 7.9-8.1; and temperature 23-25° C.

The fathead minnows, bluegills, and brown bullheads were obtained from ponds at the Newtown Fish Farm. All other species were collected from Shayler Run and nearby streams. Test fish were held in 50-gal glass aquaria receiving flowing water similar to that used during the testing. All fish were acclimated in the laboratory to the exposure temperature of $24^\circ \pm 1^\circ \text{C}$ for at least 30 days.

Bluegills and brown bullheads were larger, and only five fish per duplicate test chamber were used. All other species were tested with 10 individuals per duplicate chamber. The LC50 values were calculated by graphical analysis (American Public Health Association, 1965) for each duplicate series of 10 fish per concentration; for the bluegills and brown bullheads, the duplicates were combined for the calculation. The LC50 values and 95% confidence limits were also calculated from the data on the combined duplicates. Total copper concentrations were measured from daily grab samples. In some cases these grab samples were composited for the duration of the test, and the composite concentration was measured.

Shayler Run Water--

A series of flow-through tests was done at the NFTS for appraisal of acute copper toxicity in Shayler Run water. The dilution water was hauled over a 2- to 3-day period and stored in an underground 19-kiloliter polyester-lined tank and a 11.4-kiloliter cement cistern. The water was piped to an indoor stainless steel headbox for use during the test. Test water was aerated continuously in these tanks and in the indoor stainless steel headbox. From this headbox a pipe manifold delivered water to three proportional diluters.

For the two series of tests conducted during November 1969, the diluters delivered a control and five test concentrations to each exposure system. A dilution factor of 0.6 was used in this series. In the other tests the diluters delivered a control and six concentrations with a dilution factor of 0.5.

All diluters delivered 500 ml per cycle to the duplicate exposure chambers, which received 120-180 L per day. These glass exposure chambers were 30 cm by 60 cm by 30 cm high and were calibrated to contain 30 L. Test concentrations were randomly assigned to the chambers arranged in two rows. The source of test animals was the same as those used in the standard dilution water. For the test that started in November 1969, the fish were held in flowing standard laboratory water for 20 days and then acclimated for 10 days in Shayler Run water. For the tests started on November 15, 1970, and May 6, 1971, the fish were held at the stream in continuous-flow tanks with aeration. They were held for at least 30 days and were fed Oregon Moist fish food and frozen brine shrimp. Three days before the test they were brought into the NFTS laboratory and held in stream water to be used during the test. For the last test the fish were held in standard water for 4 months, after which the fish were held in Shayler Run water for 2 days before testing.

Because it had been shown that the acute toxicity of copper in Shayler Run water varied greatly with water quality, the species were tested simultaneously in the three exposure systems with 10 fish per test concentration. Routine chemical analyses were made as in the tests with standard water. In addition, some dissolved copper measurements were made.

In conjunction with the continuous-flow testing, a static reference bioassay was conducted using the bluntnose minnow or the fathead minnow. Duplicate series of five fish per 10 L were used in dilution water and at a temperature similar to those of the continuous-flow tests.

RESULTS

Water Quality Studies

Three series of acute toxicity tests were conducted to develop information on the variation of copper toxicity due to the varying water quality of Shayler Run. For the first series, weekly bioassays were conducted at the NFTS laboratory with stream water at laboratory temperature. The results of these static toxicity tests and characteristics of the dilution water are given in Table 20. The nominal copper LC50 values for the bluntnose minnow varied from a high of 21 mg/L to a low of 0.57 mg/L; very few fish died after 24 hr of exposure. Hardness varied from 322 mg/L (as CaCO₃) to 134 mg/L and alkalinity varied from 220 mg/L (as CaCO₃) to 98 mg/L. Both hardness and alkalinity were lower under high flow conditions.

Agreement was good between the LC50 values calculated by graphical interpolation and by probit analysis, but because only 10 fish were used per concentration with a dilution factor of 0.5, the 95% confidence limits were rather large. The test conducted on July 21 was set up with four replicates of 10 fish per concentration to check replication of results. The agreement of LC50 values of these four bioassays was excellent, but because of the lack of two partial kills, probit analysis could not be made.

Under low-flow stream conditions LC50 values were much higher than expected. Toxicity appeared to be related to stream flow; copper was less toxic (LC50 values higher) during low-flow conditions. A correlation analysis was made comparing LC50 values to stream flow, and the relationship was significant ($P=0.05$).

Because of the great variation in LC50 values, additional chemical characteristics were measured to find other variables that might be related to toxicity. The results of the second series of bioassays with the bluntnose minnow are given in Table 21. A summary of additional chemical analysis of the test water for these bioassays is given in Table 22. The stream flow during October 1969 was the lowest mean monthly flow of any month during the study. The mean daily flow never exceeded $0.017 \text{ m}^3/\text{sec}$. Despite the stability of stream flow, hardness, and alkalinity, the 24-hr LC50 values for total copper averaged 8.7 mg/L and varied from 0.75 mg/L to 22 mg/L .

Stepwise regression analysis of the 24-hr LC50 values (graphical interpolation) and the chemical and physical measurements were made. There was a significant ($P=0.01$) correlation of LC50 values with total phosphate and potassium. During this period of low stream flow the correlation coefficient for LC50 values and stream flow was not significant ($P=0.05$).

The third series of bioassays was conducted with the fathead minnow at the streamside laboratory, and the 96-hr and 7-day LC50 values are given in Table 23. A summary of additional chemical analysis of test water for these bioassays is given in Table 24. The 96-hr and 7-day LC50 values for nominal total copper varied widely, approximately 25 and 45 times, respectively, $23.6\text{--}0.92 \text{ mg/L}$ and $23.6\text{--}0.56 \text{ mg/L}$. The LC50 values based on dissolved copper, however, varied only threefold, $0.52\text{--}1.40 \text{ mg/L}$, for 96 hr and fourfold $0.36\text{--}1.40 \text{ mg/L}$, for 7 days. At temperatures above 10°C the lethal threshold for copper was apparently reached in 96 hr, since no additional deaths occurred after that time. However, at temperatures below 10°C test fish were still dying at the end of the test period of 7 days.

Higher LC50 values for both total and dissolved copper, indicating low toxicity, generally occurred during the summer and low-flow periods, whereas lower LC50 values, indicating high toxicity, occurred during the winter and spring and higher stream-flow periods. The higher LC50 values were also generally associated with higher values of measured chemical characteristics attributable to effluent from the sewage treatment plant. During low-flow periods the effluent from the treatment plant made up 80-90% of the stream flow at the gaging station.

Stepwise regression analysis of total copper 96-hr LC50 values and water quality characteristics indicated a significant ($P=0.01$) correlation of LC50 values with sodium (Na), total phosphate (TP), potassium (K), nitrite (NO_3), temperature, chlorine (Cl), total solids (TS), and conductivity. Stream flow was not significant ($P=0.05$). For dissolved copper LC50 values there was no significant ($P=0.01$) correlation with any of the water quality characteristics measured; however, TP and TS were significantly ($P=0.05$) correlated with dissolved copper LC50 values.

Two tests were made to compare copper toxicity in water from different locations in Shayler Run: upstream from the sewage treatment plant and downstream at the regular sampling station. During normal flow the downstream water had a higher hardness and alkalinity than Shayler Run water above the

TABLE 21. SUMMARY OF LC50 VALUES OF COPPER FOR THE BLUNTNOST MINNOW IN SHAYLER RUN WATER

Date	LC50 Graphical interpolation			LC50 (mg/l.) Probit analysis			Physical and chemical analysis		
	Nominal total copper		Dissolved copper	95% Confidence limits		48-hr	Instantaneous stream flow (m ³ /sec)	Hardness (mg/l. as CaCO ₃)	Alkalinity (mg/l. as CaCO ₃) pH
	24-hr	48-hr	24-hr	24-hr	48-hr				
10/7/69	6.3	6.3		6.5	4.9-9.9	6.5	0.0178	320	226 8.2
10/8/69	9.0	9.0	0.53	-	-	-	0.0204	324	226 8.2
10/9/69	4.7	4.7		4.9	3.5-6.8	4.9	0.0178	324	226 8.1
10/10/69	11	11		7.9	5.4-11.8	7.9	0.0178	320	228 8.0
10/13/69	5.7	5.7	0.42	5.7	4.2-10.7	5.7	0.0261	318	213 8.0
10/14/69	10	10	0.30	8.7	6.0-14.8	8.7	0.0241	318	210 8.0
10/15/69	8.0	8.0	0.32	9.2	6.3-13.7	9.2	0.0187	314	210 8.0
10/16/69	12	11	0.33	17.1	9.3-15.9	11.0	0.0178	318	214 7.9
10/17/69	9.7	9.7	0.42	8.3	6.2-11.2	8.3	0.0161	324	218 8.1
10/20/69	8.0	7.0		8.0	5.8-11.4	7.5	0.0153	339	218 8.1
10/21/69	14	12		14.2	10.6-28.7	12.1	0.0510	310	197 8.1
10/21/69	21	21	0.33	-	-	-	0.0269	310	214 8.1
10/22/69	22	19		-	-	-	0.0300	302	212 8.1
10/23/69	8.9	8.0		11.4	8.5-16.0	11.4	0.0241	296	198 8.0
10/27/69	11	11		8.6	6.2-12.3	7.1	0.0187	332	220 8.1
10/29/69	9.2	6.3		2.4	1.6-3.6	2.0	0.1674	340	212 8.2
11/1/69	2.0	1.5	0.37	0.72	1.1-3.0	0.66	0.0374	296	198 8.2
11/3/69	0.75	0.75		2.9	2.1-4.7	2.7	0.0490	306	189 8.1
11/5/69	2.8	2.5	0.34	1.8	1.2-2.4	1.8	0.0413	308	207 7.8
11/7/69	1.6	1.6	0.40	4.7	2.9-7.9	4.7	0.0347	314	209 8.2
11/17/69	4.0	4.0	0.39				0.0312	315	220 8.3

TABLE 22. SUMMARY OF CHEMICAL ANALYSIS OF TEST WATER IN
TESTS REPORTED IN TABLE 21

Date	Ca (mg/l.)	Mg (mg/l.)	TP (mg/l.)	K (mg/l.)	Na (mg/l.)	Cl (mg/l.)	Total organic carbon (mg/l.)	NO ₃ - N (mg/l.)
10/7/69	93	20.3	7.4	10.0	71	80	5.4	10.5
10/8/69	96	20.7	7.6	10.6	71	76	5.4	10.0
10/9/69	96	20.3	7.4	10.5	71	80	6.2	10.4
10/10/69	96	20.2	8.0	10.4	73	89	6.4	10.2
10/13/69	93	20.0	7.3	11.9	72	77	6.4	9.9
10/14/69	93	20.0	8.0	11.7	68	77	6.2	10.1
10/15/69	93	20.0	7.8	11.1	66	74	7.0	9.7
10/16/69	93	20.3	7.6	9.5	70	80	5.8	9.4
10/17/69	93	20.3	7.8	9.6	68	82	6.0	9.6
10/20/69	94	21.4	7.6	9.8	72	87	5.8	11.2
10/21/69	89	19.3	9.0	13.2	67	81	8.6	11.7
10/21/69	89	19.4	9.3	13.4	66	73	8.4	10.6
10/22/69	88	19.2	8.9	12.9	62	68	7.0	10.0
10/23/69	81	18.7	8.4	11.3	57	61	8.6	10.7
10/27/69	90	20.9	7.7	10.9	62	68	5.2	12.5
10/29/69	92	20.9	7.9	10.8	60	71	6.8	12.6
11/1/69	83	19.0	7.1	11.0	63	71	7.6	10.5
11/3/69	78	18.0	4.8	8.0	38	41	7.4	5.3
11/5/69	83	19.9	5.8	8.3	47	54	6.2	7.0
11/7/69	85	19.8	5.5	8.7	53	59	6.0	7.3
11/17/69	87	20.4	5.3	7.8	50	53	4.8	8.8

TABLE 23. SUMMARY OF STATIC BIOASSAY WITH FATHEAD MINNOWS IN SHAYLER RUN WATER

Date	Instantaneous stream flow m ³ /sec	Hardness (mg/l, as CaCO ₃)	Alkalinity (mg/l, as CaCO ₃)	Temperature range °C	Control pH	Nominal copper		pH ^b	Dissolved copper	
						96-hr LC50 (mg/l, x)	168-hr LC50 (mg/l, x)		96-hr LC50 (mg/l, x)	168-hr LC50 (mg/l, x)
2/25/70	0.143	280	190	4-7	8.0	4.9	2.6	8.0	-	-
3/11/70	0.165	280	184	2-4	8.0	3.3	1.7	8.0	0.73	0.38
3/25/70	0.170	244	160	5-8	8.2	1.6	1.2	8.0	0.73	0.63
4/1/70		212	132	7-12	8.3	2.0	1.6	8.5	0.66	0.39
4/8/70	0.199	260	170	10-15	8.1	3.5	2.3	8.0	0.90	0.67
4/13/70	0.111	402	198	12-17	8.0	16	13	-	0.80	0.80
4/23/70	0.139	174	160	15-21	8.0	8.1	0.9	8.0	1.06	0.90
4/30/70	0.120	228	138	12-21	8.1	1.0	0.80	7.9	0.82	0.80
5/13/70	0.037	110	96	19-29	8.1	2.8	2.8	7.9	0.94	0.94
5/20/70	0.073	110		16-21	8.2	9.0	9.0	7.1	0.81	0.76
5/21/70	0.023	308	210	22-30	8.3	8.7 ^a	12.7 ^a	7.2	0.97 ^a	20.82 ^a
5/28/70	0.014	136	200	20-27	8.3	21	13	7.3	0.80	0.80
5/26/71	0.039	280	180	10-21	8.3	13	7	7.5	0.78	0.73
5/28/70	0.029	280	180	24-27	8.2	10	9.5	7.3	0.76	0.69
5/29/70	0.027	266	170	22-27	8.4	35	18.5	7.8	0.60	0.61
8/19/70	0.035	110	206	20-25	8.4	19 ^a	12.5 ^a	7.9	0.81 ^a	0.81 ^a
9/9/70	0.026	124	220	18-24	8.1	18 ^a	18.3 ^a	7.9	0.78 ^a	0.78 ^a
9/30/70	0.026	290	172	13-13	8.2	3.17 ^a	2.21 ^a	7.9	0.49 ^a	0.41 ^a
10/13/70	0.170	260	184	8-14	7.9	22.7	22.1	7.8	1.09	1.09
10/28/70	0.060	240	132	11-19	8.2	14.5 ^a	14.5 ^a	7.5	0.96 ^a	0.92 ^a
11/27/70	0.110	242	170	9-17	8.0	0.60	0.65	8.1	0.65	0.63
12/7/70	0.034	308	210	10-13	8.0	4.83	1.19	7.8	0.73	0.63
1/6/71	0.237	306	140	1-3	8.2	0.82	0.36	8.2	0.60	0.44
1/20/71	0.044	262	190	7-7	8.1	1.19	0.40	8.1	0.68	0.38
2/13/71	0.333	322	242	1-3	8.0	2.83	1.41	8.0	0.92	0.38
2/17/71	1.98	210	130	1-1	8.1	1.40	0.89	8.0	0.64	0.48
3/4/71	0.139	260	174	1-8	8.1	1.38	0.81	7.9	0.82	0.58
3/25/71	0.139	250	180	2-11	7.8	1.00	0.33	7.8	0.38	0.38
4/22/71	0.039	312	212	10-19	8.1	0.11	0.00	7.8	0.76	0.73
5/13/71	0.208	272	200	11-26	8.3	1.00	1.00	8.1	0.38	0.38
5/18/71	0.096	276	208	14-23	8.2	3.16	1.63	8.0	0.69	0.69
6/22/71	0.061	284	208	20-28	8.1	8	2.5	-	0.63	0.63
6/26/71	0.088	252	176	20-28	8.1	10.5	10.5	7.6	0.63	0.63
6/30/71	0.023	298	210	22-28	8.2	22.7	22.7	7.8	1.40	1.20
7/14/71	0.077	292	188	21-29	8.1	21.8	21.8	7.3	0.96	0.96
7/28/71	0.027	284	194	18-24	8.2	23.6	23.6	7.6	0.60	0.79

^a Possibly dissolved greater than expected due to low concentrations - value used in data analysis.

^b pH for water in test concentration nearest to LC50.

TABLE 24. CHEMICAL ANALYSIS OF TEST WATER IN TESTS REPORTED IN TABLE 23

Date	Loc.	Total solids (mg/l.)	Dissolved solids (mg/l.)	Specific conductance (microhm)	Total organic carbon (mg/l.)					Total N (mg/l.)	Si (mg/l.)	P (mg/l.)	Ca (mg/l.)	Mg (mg/l.)
					NO ₃ -N (mg/l.)	NO ₂ -N (mg/l.)	NO ₃ -N (mg/l.)	NO ₂ -N (mg/l.)	NO ₃ -N (mg/l.)					
7/25/70	1-1	459	440	651	1.3	0.2	0.8	0.2	0.8	16	4.4	36	750	11
7/31/70	1-2	389	384	659	1.6	0.2	0.8	0.2	1.1	26	3.8	34	75	11
8/13/70	0-7	345	340	604	1.0	0.3	0.3	0.0	0.8	19	3.2	34	40	11
8/17/70	0-4	291	290	429	0.8	0.3	0.3	0.0	0.3	19	1.5	32	40	11
8/17/70	1-1	70	318	322	1.2	0.1	0.1	0.0	0.8	21	4.9	33	80	14
9/15/70	1-4	408	372	689	0.1	0.1	1.3	0.0	0.1	28	3.2	38	84	11
9/20/70	1-1	358	340	496	0.8	0.1	0.1	0.8	0.8	22	3.0	33	68	14
10/14/70	0-8				0.5	0.1	0.9	0.0	0.0	14	3.1	30	61	12
10/20/70	0-4	308	292	606	1.2	0.2	0.3	0.0	0.0	24	3.4	31	80	11
11/1/70	1-2	478	461	624	0.9	0.1	0.3	0.8	0.5	27	3.3	33	80	18
1/26/71	1-1	618	609	911	2.4	0.1	0.3	0.9	0.8	69	3.1	40	80	21
1/26/71										45	3.1	30	18	11
1/27/71										30	3.9	61	61	11
1/28/71										0	3.8	30	18	11
1/28/71	0-8	117	454	742	1.0	0.1	0.1	0.0	0.3	0	3.4	30	60	18
8/19/70	0-1	333	331	431	0.9	0.1	0.1	0.8	0.8	30	3.4	36	60	18
8/19/70	1-4	648	600	916	2.8	0.1	0.3	0.0	1.8	20	3.8	32	60	11
8/30/70	0-0	306	292	480	0.9	0.1	0.1	0.0	0.0	33.4	3.3	36	60	18
10/14/70	1-4	478	461	570	1.9	0.1	0.0	0.0	0.1	11.1	3.3	38	30	11
10/20/70	0-3	334	329	677	0.8	0.1	0.4	0.4	0.0	0.1	3.0	30	60	11
11/21/70	0-4	374	363	602	0.1	0.0	0.3	0.0	0.0	11.1	3.0	30	60	11
11/21/70	0-8	328	317	504	1.1	0.1	0.8	0.0	0.1	21.9	3.0	31	68	11.1
1/10/71	0-1	401	415	813	1.2	0.1	0.4	0.0	0.4	2.8	3.0	30	30	11.1
5/1/71	0-8	290	291	406	0.8	0.1	0.8	0.0	0.0	3.0	3.0	30	30	11.1
1/11/71	0-3	401	371	596	1.2	0.1	0.6	0.8	0.0	1.7	3.4	30	30	11.1
3/2/71	0-7	405	393	656	0.8	0.3	0.9	0.0	1.2	3.1	3.0	30	30	11.1
3/2/71	0-9	330	318	464	0.7	0.1	1.0	0.0	0.1	1.1	2.0	30	30	11.1
4/11/71	1-4	430	431	708	1.1	0.1	0.1	0.0	0.1	0.0	3.0	30	30	11.1
5/13/71	1-2	426	412	621	1.4	0.1	0.4	0.0	0.0	0.0	3.0	30	30	11.1
5/14/71	1-1	433	415	638	1.2	0.1	0.4	0.0	0.0	0.0	3.0	30	30	11.1
6/1/71					0.4	0.2	0.7	0.8	0.0	0.0	3.0	30	30	11.1
6/16/71	1-8	460	426	671	0.9	0.2	0.8	0.0	0.0	1.6	3.0	30	30	11.1
6/30/71	0-1	369	340	666	0.0	0.1	0.1	0.8	0.0	0.1	3.0	30	30	11.1
7/14/71	0-8	388	392	785	2.0	0.1	0.3	0.0	1.8	1.9	3.0	30	30	11.1
1/26/71	0-8	1,340	534	920	2.0	0.1	0.2	1.4	0.7	0.0	3.0	30	30	11.1

sewage treatment plant (Table 25). Bioassays were conducted in unmodified upstream and downstream water. In addition, the harder downstream water was diluted with demineralized water to prepare a modified water similar in hardness to the upstream water. The first bioassay gave total LC50 values of less than 1.0 mg/L of copper in the upstream water and the diluted and softened downstream water (Table 25); the lowest concentration tested was 1.0 mg/L. A similar test was conducted with water collected July 29, 1969. The bioassay in unmodified downstream water gave an LC50 value of 8.0 mg/L. (Table 25). The LC50 values in the upstream water and in the diluted downstream water were 0.35 and 0.50 mg/L., respectively.

Additional tests were made to evaluate the effect of dilution of Shayler Run water on copper toxicity. For the first test, demineralized water was used to prepare three dilutions of stream water. The 24-hr LC50 values for copper ranged from 19 mg/L. for unmodified stream water to 3.6 mg/L. for the greatest dilution (Table 26). Bioassays in the intermediate dilutions had similar LC50 values of 10 and 9.1 mg Cu/L. This dilution not only lowered total hardness and alkalinity, but also diluted other detoxifying agents in the stream water. The LC50 value of 3.6 mg/L. was much higher than would have been predicted since the standard water (200 mg/L. hardness) gave an LC50 value of 0.45 indicating that other detoxifying agents were present. At the low flow of this sampling period, most of the flow was due to the flow from the sewage treatment plant.

The second test, September 18, 1969, was conducted in stream water collected at a flow of 0.085 m³/sec and falling from a high flow of 0.170 m³/sec. Thus, there was much dilution of the sewage treatment plant effluent. For this test the stream water was diluted with a 1-to-1 dilution of demineralized water with a reconstituted water similar in hardness and alkalinity to the stream water. The LC50 value in the unmodified stream water was 17 mg/L. of copper. In the stream water diluted with reconstituted water, the LC50 value was 0.78 mg/L., and copper was even more toxic in the stream water diluted with demineralized water. Apparently the rain had greatly reduced the concentration of detoxifying agents contributed by the sewage treatment plant, and the copper was much more toxic in this dilution water than in the first test.

A dilution water containing calcium chloride, magnesium sulfate, and sodium bicarbonate in demineralized water was prepared with similar hardness, alkalinity, and pH as the stream water. The stream water was diluted with this water to 75%, 50%, and 25% stream water. Toxicity increased with decreasing amounts of Shayler Run water. The LC50 values varied from 11 mg/L. in the unmodified stream water to 2.9 mg/L. in the test water containing the smallest percentage of Shayler Run water. These tests indicated that some chelating agent or agents from the sewage treatment plant were being diluted, and thus copper toxicity was increased.

Three tests were conducted to determine the effect on copper toxicity of added hardness at a constant alkalinity. These results are summarized in Table 27. Calcium chloride and magnesium sulfate were used to maintain the calcium-magnesium ratios in Shayler Run water. The first test was conducted in rain-diluted Shayler Run water. Of all the unmodified stream water bioassays, copper was the most toxic in this dilution water, with an LC50 value of 0.57 mg/L. The LC50 value of 0.76 mg/L. indicated, at most, only minor effect of

TABLE 25. TOTAL COPPER LC50 VALUES FOR THE BLUNTNOSE MINNOW IN SHAYLER RUN
WATER UPSTREAM AND DOWNSTREAM FROM THE SEWAGE TREATMENT PLANT

Date	Source of dilution water	Hardness (mg/l.) as CaCO ₃	Calcium (mg/l.)	Magnesium (mg/l.)	Alkalinity (mg/l. as CaCO ₃)	24-hr LC50 (mg/l.)
7/25/69	Upstream	173	48	10.6		<1.0
	Downstream, unmodified	268	75	15.6		6.0
	Downstream, diluted	175				<1.0
7/29/69	Upstream	196	53	131	156	0.35
	Downstream, unmodified	300	82.5	19.2	220	8.0
	Downstream, diluted	196			148	0.50

TABLE 26. TOTAL COPPER LC50 VALUES FOR THE BLUNTNOSE MINNOW IN VARIOUS DILUTIONS OF SHAYLER RUN WATER

Date	Test water	Graphical interpolation (LC50 mg/l.)		Probit analysis LC50 (mg/l.)		Hardness (mg/l., as CaCO ₃)	Ca (mg/l.)	Mg (mg/l.)	Alkalinity (mg/l., as CaCO ₃)	pH
		24-hr	48-hr	48-hr	95% confidence limits					
8/4/69	Unmodified Shayler Run water	19	19	17.3	14.5-20.6	316	83	18.6	206	7.8
	Shayler Run water diluted with demineralized water									
	Nominal hardness (mg/l., as CaCO ₃)									
	250	10	9.6	7.7	6.2-9.7	255	69	13.2	174	7.8
9/18/69	50% Shayler Run water	9.1	9.1	6.8	5.2-9.0	295	53	11.5	138	7.8
	50% Reconstituted water									
	50% Shayler Run water	3.6	3.3	3.2	2.4-5.6	138	43	10.0	110	7.7
	50% Reconstituted water									
9/3/69	Unmodified Shayler Run water	17	17			222	54	14.8	170	
	50% Shayler Run water	0.78	0.78			220			176	
	50% Reconstituted water									
	50% Shayler Run water	0.25	0.25			112			90	
9/3/69	50% Reconstituted water									
	Unmodified Shayler Run water	11	11			292	81	18.3	204	7.9
	Shayler Run water diluted with reconstituted water									
	75% Stream water	6.7	6.7			300			204	7.9
9/3/69	50% Stream water	3.6	3.6			302	81.7	19.5	200	8.0
	25% Stream water	2.9	2.9			296	81	19.5	202	7.9

TABLE 27. EFFECT OF HARDENING SHAYLER RUN WATER ON THE TOXICITY OF
TOTAL COPPER TO THE BLUNTNOSSE MINNOW

Date	Dilution water	Graphical interpolation LC50 (mg/l.)		Probit analysis LC50 (mg/l.)				Hardness (mg/l. as CaCO ₃)	Alkalinity (mg/l. as CaCO ₃)	pH
		24-hr	48-hr	24-hr	confidence limits	48-hr	95% confidence limits			
8/20/69	Unmodified stream water	0.57	0.57	0.66	0.47-0.95	0.66	0.47-0.95	170	150	7.9
	Modified stream water									
	210 hardness ^a	0.85	0.85	0.94	0.66-1.3	0.94	0.66-1.3	234	146	7.9
8/21/69	295 hardness	1.7	1.6	1.6	1.1-2.3	1.6	1.1-2.3	302	144	7.9
	Unmodified stream water	3.0	3.0	3.0	2.2-4.1	3.0	2.2-4.1	218	180	8.1
	Modified stream water	4.0	3.5	3.7	2.7-4.9	3.4	2.5-4.6	284	178	8.0
8/25/69	Unmodified stream water	9.2	9.2					276	216	8.0
	Modified stream water									
	320 hardness	9.2	9.2					310	208	7.9
	370 hardness	8.0	8.0					370	210	8.0

^aHardness expressed in mg/l. as CaCO₃.

added calcium and magnesium. For the bioassay of August 21, 1969, the stream flow had fallen to 0.059 m³/sec, with a corresponding hardness of 218 mg/L (as CaCO₃). The bioassay in the unmodified stream water gave an LC50 value of 3.0 mg/L. With added calcium and magnesium the LC50 value was 4.0 mg/L. For the third test the stream flow had fallen to 0.02 m³/sec. The LC50 value in the unmodified water was 9.2 mg/L. In the intermediate hardened water of 330 mg/L, a similar LC50 value of 9.2 mg/L was obtained. In the hardest water the LC50 value was lower--8.0 mg/L. This lower value resulted from the death of one more test fish in the test concentration of 8.0 mg/L. These three toxicity tests indicate that added hardness (calcium and magnesium) had only a small effect on the toxicity of copper in Shayler Run water. Apparently the reduction of copper toxicity at lower stream flows was due to the greater contribution of agents in the effluent from the sewage treatment plant during this time and not to variation in alkalinity or hardness.

A supplemental test was conducted with standard water to evaluate the effect of added calcium and magnesium. This standard water was diluted with demineralized water, and calcium and magnesium salts were added to obtain test waters of different hardness but similar alkalinity. In addition, the regular standard water was used. The LC50 values in the dilution water with a constant alkalinity ranged from 0.15 mg/L in the lowest hardness water to 0.26 mg/L in the higher hardness dilution waters (Table 28). In the standard water with an alkalinity of 154 mg/L, the LC50 value was 0.29 mg/L. These tests in standard laboratory dilution water indicated that the great variation in acute toxicity values obtained from Shayler Run water could not be explained in terms of hardness.

Toxicity tests were carried out to determine the effect of added phosphate on the toxicity of copper to the bluntnose minnow (Table 29). In the first test 7.6 mg P/L were added as dibasic sodium phosphate to stream water. The total copper LC50 value in unmodified stream water was 5.6 mg/L, and in the bioassay conducted with the added phosphate the copper was less toxic with an LC50 value of 20 mg/L (Table 29). In the second test bioassays were conducted with unmodified stream water containing 7.4 mg/L total phosphate to which 0, 125, and 500 mg/L of pyrophosphate were added. Copper was less toxic in the water with the added phosphate and was still less toxic in the higher concentration of phosphate. The third test was conducted in standard water, which is low in total phosphate (0.2 mg/L or less). Again, copper was less toxic in the water with added pyrophosphate. The difference in the LC50 value for total copper was greater than tenfold between the standard water and the water with the higher concentration of added pyrophosphate. Dissolved copper measurements were made, and the range in LC50 values of dissolved copper was about as great as the range for total-copper LC50 values. Apparently much of the pyrophosphate-copper complex goes through the 0.45-millipore filter. However, the toxicity of this complex appears to be greatly reduced.

Bluntnose minnows were brought into the laboratory on August 13, 1969. They were held and tested in standard water. Five toxicity tests over a 2-month period showed that the sensitivity of these fish to copper did not change with being held in the laboratory. The LC50 values were similar in all tests (Table 30).

TABLE 28. EFFECT OF HARDENING STANDARD WATER ON THE ACUTE TOXICITY OF TOTAL COPPER TO THE BLUNTNOSE MINNOW

Date	Dilution water	LC50 values (mg/l.)		Hardness (mg/l. as CaCO ₃)	Alkalinity (mg/l. as CaCO ₃)	Ca (mg/l.)	Mg (mg/l.)	pH
		24-hr	48-hr					
8/29/69	Unmodified standard water - 200 hardness ^a	0.29	0.29	208	154			7.7
	Modified standard water							
	125 hardness ^b	0.15	0.15	132	100	25	15.5	7.7
	175 hardness ^c	0.20	0.20	182	99			
	225 hardness ^c	0.18	0.18	233	99	44	27.3	7.6
	275 hardness ^c	0.26	0.26	282	99			7.6
	325 hardness ^c	0.26	0.26	337	98	64	36.1	7.7

^aHardness expressed in mg/l. as CaCO₃.

^b200 hardness diluted with demineralized water to 125 hardness.

^cCalcium chloride and magnesium sulfate added to 125 hardness water in the same ratio of Ca/Mg.

TABLE 29. EFFECT OF ADDED PHOSPHATE ON THE LC50 OF COPPER TO THE BLUNTNOSE MINNOW

Date	Test conditions	Graphical interpolation LC50 (mg/l.)				Probit analysis LC50 (mg/l.)				Dilution water		
		Total copper		Dissolved copper		Total copper		95% confidence limits		Hardness (mg/l. as CaCO ₃)	Alkalinity (mg/l. as CaCO ₃)	pH
		24-hr	48-hr	24-hr	48-hr	24-hr	48-hr	24-hr	48-hr			
9/9/69	Unmodified Shayler Run water	5.6	5.6			4.5	4.5	3.0-6.9	3.0-6.9	220	172	7.9
	(Na ₂ HPO ₄ ·7 H ₂ O added; 7.7 mg/l., total phosphorus	20	20			14.7	14.7	10.2-22.7	10.2-22.7			
10/7/69	Unmodified Shayler Run water	6.3	6.3							322	212	7.9
	(Na ₂ P ₂ O ₇ ·10 H ₂ O added)											
	17.4 mg/l., total phosphorus 69.5 mg/l., total phosphorus	11 25	11 25			10.4	9.7	7.4-14.8 22.9-41.6	7.0-13.3 22.9-41.6			
10/17/69	Standard water - 200 mg/l. hardness	0.16	0.16	0.15						203	171	8.2
	(Na ₂ P ₂ O ₇ ·10 H ₂ O added)											
	9.24 mg/l., total phosphorus 37.8 mg/l., total phosphorus	1.1 2.9	1.1 2.9	0.82 1.3								

TABLE 30. ACUTE TOXICITY TO THE BLUNTNOSE MINNOW
OF COPPER IN STANDARD WATER

Date	LC50 values (mg/L.)		
	24-hr	48-hr	96-hr
8/29/69	0.29	0.29	0.29
9/16/69	0.26	0.26	0.26
10/2/69	0.29		
10/9/69	0.26	0.26	0.26
10/27/69	0.28	0.28	0.28

Relative Sensitivity Studies

The results of copper-toxicity tests with standard water on 10 fish species are summarized in Table 31. The 24-, 48-, and 96-hr LC50 values of measured total copper are given for graphical interpolation, and, in addition, the 96-hr LC50 value and 95% confidence limits were obtained by using probit analysis on the data of the combined duplicates. There were no partial kills in either bluegill test, so it was not possible to use our program of probit analysis. Of the 10 species tested the bluegill was the most resistant. The striped shiner and the orangethroat darter were more sensitive than the bluegill, but more resistant than the other seven species. Agreement was excellent between the two methods of calculating LC50 values, based on 20 test fish per concentration and a dilution factor of 0.6.

The LC50 values obtained in the first series of tests with Shayler Run water are shown in Table 32. The 96-hr LC50 values (graphical interpolation) varied from 4.8 mg/L. for the rainbow darter to 11 mg/L. for the fathead minnow. The measured LC50 values were about 10 times the values for those species tested in the standard water (hardness, 200 mg/L. as CaCO_3). However, the dissolved copper values were similar to the total copper values found in the standard water. At concentrations of less than 1 mg Cu/L. in the standard water, we found that about 85% of the total copper was "dissolved." The dissolved copper LC50 of 0.38 found in the static reference bioassay was similar to the value in this flow-through test.

A week later the preceding test was repeated with fish from the same stock. Hardness, alkalinity, pH, and temperature of the dilution water were similar in this and the preceding series of bioassays. In all cases the LC50 values for each species were higher for this test than in the preceding test (Table 33). Also, the LC50 value of the bluntnose minnow in the static test was higher. In both tests the rainbow darter was the most sensitive species, and the striped shiner and fathead minnow were the most resistant. For this test the LC50 values of the orangethroat darter, brown bullhead, and bluntnose minnow were all within the confidence limits of these three species.

The results of the toxicity test started on December 15, 1970, are given in Table 34; some of the results of copper analyses for this test are given in Table 35. The bluegill was the most resistant species, and the stoneroller was the most sensitive. The 96-hr LC50 values for both total and dissolved copper were over 10 times higher for the bluegill than for the stoneroller. On the basis of 96-hr LC50 values for total copper, the rainbow darter was more sensitive than the orangethroat darter, and their confidence limits did not overlap. However, their 96-hr LC50 values for dissolved copper were not different.

The acute toxicity of copper in Shayler Run water for the test started on May 6, 1971, is summarized in Table 36. The results of the copper analysis are given in Table 37. The 96-hr LC50 (total copper) value ranged from 16 mg/L. for the bluegill to about 5 mg/L. for the striped shiner. The LC50 value (dissolved copper) was lower for the rainbow darter than for the orangethroat darter. The phosphate concentration was higher in this test water than in the water of the preceding or following test. The static reference test gave a high 96-hr LC50 value of 19 mg/L.

TABLE 31. RELATIVE SENSITIVITY OF DIFFERENT SPECIES OF FISH TO COPPER IN STANDARD WATER

Species	Date	Duplicate	LC50 - (mg/l.) measured total copper Graphical interpolation			LC50	Probit analysis - 96-hr 95% confidence limits	Average length of test fish (mm)	Average weight of test fish (g)
			24-hr	48-hr	96-hr				
Stoneroller	5/20/69	A B	0.40 0.40	0.36 0.37	0.31 0.31	0.29	0.25-0.29	60	-
Creek chub	5/26/69	A B	0.48 0.48	0.37 0.38	0.33 0.29	0.31	0.27-0.35	64	4.0
Rainbow darter	5/20/69	A B	0.40 0.46	0.34 0.40	0.34 0.36	0.32	0.26-0.38	41	-
Blacknose dace	5/26/69	A B	0.70 0.52	0.46 0.38	0.33 0.33	0.32	0.28-0.36	47	1.1
Bluntnose minnow	5/12/69	A B	0.26 0.40	0.26 0.31	0.26 0.31	0.34	0.29-0.40	84	6.7
Fathead minnow	6/16/69	A B	0.58 0.76	0.58 0.75	0.42 0.47	0.44	0.38-0.52	56	1.6
Fathead minnow	9/15/69	A B	0.60 0.52	0.60 0.47	0.55 0.42	0.49	0.41-0.59	47	-
Brown bullhead	10/8/69	A B	0.65 0.65	0.65 0.65	0.52 0.52	0.54	0.43-0.77	39	-
Striped shiner	4/1/69	A B	0.89 0.83	0.83 0.79	0.83 0.76	0.79	0.67-0.92	55	-
Orangethroat darter	6/2/69	A B	1.1 0.93	1.0 0.93	1.0 0.71	0.85	0.75-0.98	44	0.9
Striped shiner	5/12/69	A B	1.6 2.0	1.6 2.0	1.6 2.0	1.9	1.6-2.2	55	1.7
Bluegill	9/21/69		13	13	8.3			103	18.6
Bluegill	9/29/69		14	12	10			101	19.2

TABLE 32. RELATIVE SENSITIVITY OF SIX SPECIES OF FISH TO COPPER IN SHAYLER RUN WATER, ^a NOVEMBER 12, 1969

Species	Graphical interpolation			Probit analysis			Average length of test fish (mm)	Average weight of test fish (g)		
	LC50		Measured dissolved copper (mg/l.)	96-hr LC50		Measured total copper (mg/l.)				
	24-hr	48-hr		96-hr	95% confidence limits					
									LC50	G(Heterogeneity factor)
Rainbow darter	14	5.9	4.6	<0.36	5.2	4.2-6.4	0.31	46		
Brown bullhead		11.1	5.8	5.2	<0.36	5.1	3.9-6.1	0.34	52	1.4
Bluntnose minnow ^b		10	10	6.8	0.39	7.3	5.9-8.8	0.21	39	0.43
Orangethroat darter		9.9	9.9	7.1	<0.72				44	
Striped shiner		9.4	8.4	8.4	0.72				47	
Fathead minnow		13	11	11	0.34	9.6	7.8-12	0.20	44	

^a Dilution water - hardness = 314 mg/l., as CaCO₃; alkalinity = 206 mg/l., as CaCO₃; pH = 8.0; test temperature = 24 °C.

^b Static bioassay - 96-hr LC50 = 16 mg/l., (nominal), 0.38 mg/l., (dissolved).

TABLE 33. RELATIVE SENSITIVITY OF SIX SPECIES OF FISH TO COPPER IN SHAYLER RUN WATER, ^a NOVEMBER 19, 1969

Species	Graphical interpolation			Probit analysis			Average weight of test fish (g)		
	LC50		Measured dissolved copper (mg/l.)	96-hr LC50		Average length of test fish (mm)			
	Measured total copper (mg/l.)			Measured total copper (mg/l.)					
	24-hr	48-hr		96-hr	LC50			95% confidence limits	G(Heterogeneity factor)
Rainbow darter	18	12	5.3	<0.59	5.5	3.1-6.9	0.46	46	1.0
Orangethroat darter	20	17	9.4	<0.59	9.8	7.8-12	0.25	44	0.8
Brown bullhead	16	15	12	0.57	11	8.1-13	0.32	53	1.5
Bluntnose minnow ^b	15	15	13	0.62	11	8.6-14	0.26	40	0.6
Fathead minnow	16	16	15	1.0	13	11-17	0.29	42	0.6
Striped shiner	16	16	16	1.1				50	0.9

^a Dilution water - hardness = 303 mg/l. as CaCO₃; alkalinity = 206 mg/l. as CaCO₃; pH = 8.0; test temperature = 24 °C.

^b Static bioassay - 11/21--96-hr LC50 = 20 mg/l. (nominal), 0.45 mg/l. (dissolved).

11/23--96-hr LC50 = 18 mg/l. (nominal), 0.47 mg/l. (dissolved).

TABLE 34. RELATIVE SENSITIVITY OF SIX SPECIES OF FISH TO COPPER IN SHAYLER RUN WATER, ^a DECEMBER 15, 1970

Species	Lepomis macrochirus			Lepomis gibbosus			Lepomis microlophus			Lepomis cyanichthys			Lepomis gibbosus			Lepomis macrochirus		
	Survival (%)	Mean length (mm)	Mean weight (g)	Survival (%)	Mean length (mm)	Mean weight (g)	Survival (%)	Mean length (mm)	Mean weight (g)	Survival (%)	Mean length (mm)	Mean weight (g)	Survival (%)	Mean length (mm)	Mean weight (g)	Survival (%)	Mean length (mm)	Mean weight (g)
Control	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
0.01 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
0.05 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
0.1 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
0.5 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
1.0 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
5.0 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
10.0 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
50.0 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0
100.0 ppm	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0	100	50.0	1.0

TABLE 35. COPPER CONCENTRATIONS (IN MILLIGRAMS PER LITER) IN EXPOSURE CHAMBER FOR TESTS REPORTED IN TABLE 34^a

Nominal concentration	24-hr Sample				72-hr Sample			
	Total Cu		Dissolved Cu ^b		Total Cu		Dissolved Cu ^b	
	A	B	A	B	A	B	A	B
Control	0.017	-	0.011	-	0.010	-	0.023	-
0.125	0.088	0.092	0.078	0.081	0.089	0.092	0.091	0.095
0.250	0.236	0.216	0.198	0.191	0.240	0.233	0.205	0.199
0.500	0.436	0.444	0.269	0.268	0.458	0.450	0.278	0.272
1.00	0.893	0.880	0.351	0.348	0.937	0.981	0.361	0.372
2.00	1.76	1.76	0.438	0.422	1.84	1.87	0.457	0.462
4.00	0.450	3.63	0.555	0.587	3.70	3.70	0.522	0.530
	C	D	C	D	C	D	C	D
Control	0.014	-	0.009	-	0.010	-	0.020	-
2.00	1.11	1.12	0.386	0.408	0.841	0.841	0.887	0.394
4.00	2.70	2.78	0.573	0.573	2.60	2.69	0.530	0.535
8.00	4.31	3.98	0.649	0.663	4.37	4.02	0.596	0.609
16.00	6.47	6.17	0.931	0.909	6.33	6.50	0.849	0.863
32.0	11.0	10.9	1.99	2.12	9.92	11.9	1.88	2.02
64.0	23.6	37.3	11.2	12.0	23.1	120.0	10.1	10.6
	E	F	E	F	E	F	E	F
Control	0.011	-	0.008	-	0.007	-	0.012	-
0.50	0.364	0.346	0.228	0.233	0.341	0.332	0.229	0.208
1.00	0.884	0.876	0.299	0.300	0.863	0.854	0.308	0.322
2.00	2.02	1.97	0.373	0.369	2.00	1.89	0.390	0.385
4.00	3.68	3.70	0.503	0.503	3.77	3.74	0.470	0.470
8.00	7.15	7.32	0.649	0.617	6.96	7.13	0.583	0.578
16.00	9.94	9.73	0.943	0.931	11.9	12.0	0.888	0.849

^a Grab samples were collected 24 and 72 hr after test began. Three diluters were used in duplicate; A and B, C and D, E and F.

^b The part that passes through a 0.45-micron filter.

TABLE 36. RELATIVE SENSITIVITY OF EIGHT SPECIES OF FISH TO COPPER IN SHAYLER RUN WATER, ^a MAY 6, 1971

Species	Duplicate	Extrapolated interpolation				Predict analysis			
		LC50		LC50		Measured total copper ($\mu\text{g/l}$)	Measured dissolved copper ($\mu\text{g/l}$)	95% confidence limits	G(heterogeneity factor)
		Measured total copper ($\mu\text{g/l}$)	Measured dissolved copper ($\mu\text{g/l}$)	Measured total copper ($\mu\text{g/l}$)	Measured dissolved copper ($\mu\text{g/l}$)				
Striped shiner	A	7.1	7.1	7.1	0.83	4.0	0.05-8.8	0.87	0.15
	B	5.1	5.1	5.1	0.77	4.0	0.05-8.8	0.87	0.15
Bluntnose minnow	A	8.3	8.0	6.8	0.73	3.0	0.06-12	0.18	0.17
	B	8.0	8.0	4.4	0.66	3.0	0.06-12	0.18	0.17
Orangebreast darter	A	8.1	6.1	3.3	0.86	3.3	0.05-8.8	0.23	0.13
	B	8.3	6.1	5.6	1.1	3.3	0.05-8.8	0.23	0.13
Johnny darter	A	9.3	8.0	6.8	0.83	3.3	0.05-8.8	0.23	0.13
	B	10	8.0	8.0	0.86	3.3	0.05-8.8	0.23	0.13
Rainbow darter	A	12	10	10	1.1	3.3	0.05-8.8	0.23	0.13
	B	10	10	10	0.93	3.3	0.05-8.8	0.23	0.13
Fathead minnow	A	12	12	12	1.1	3.3	0.05-8.8	0.23	0.13
	B	11	11	11	1.0	3.3	0.05-8.8	0.23	0.13
Crack chub	A	16	16	16	2.3	3.3	0.05-8.8	0.23	0.13
	B	16	16	16	2.3	3.3	0.05-8.8	0.23	0.13
Bluntnose	A	16	16	16	2.3	3.3	0.05-8.8	0.23	0.13
	B	16	16	16	2.3	3.3	0.05-8.8	0.23	0.13

^a Diffusion water - hardness = 116 mg/l, as CaCO_3 ; alkalinity = 214 mg/l, as CaCO_3 ; pH = 6.2; Test temperature = 19 °C.
 Static bioassay - 96-hr LC50 = 19 mg/l, (nominal); 1.3 mg/l, (dissolved).

TABLE 37. COPPER CONCENTRATIONS (IN MILLIGRAMS PER LITER) IN EXPOSURE CHAMBER FOR TESTS REPORTED IN TABLE 36^a

Nominal concentration	24-hr Sample				96-hr Sample			
	Total Cu		Dissolved Cu ^b		Total Cu		Dissolved Cu ^b	
	A	B	A	B	A	B	A	B
Control	0.011	-	-	-	0.010	-	-	-
0.125	0.072	-	0.087	-	0.090	-	0.090	-
0.250	0.189	-	0.200	-	0.216	-	0.208	-
0.50	0.356	-	0.316	-	0.416	-	0.297	-
1.0	0.777	-	0.438	-	0.829	-	0.400	-
2.0	1.533	1.533	0.533	-	1.680	1.680	0.494	-
4.0	3.000	-	0.608	-	3.400	-	0.530	-
	C	D	C	D	C	D	C	D
Control	0.010	-	-	-	0.013	-	-	-
2.0	1.118	1.229	0.411	-	0.753	0.847	0.370	-
4.0	4.889	-	0.600	-	2.625	-	0.541	-
8.0	5.286	-	0.672	-	4.806	-	0.595	-
16.0	6.023	-	0.980	-	6.240	-	0.892	-
32.0	10.200	-	2.128	-	11.540	-	1.811	-
64.0	23.240	-	-	-	22.690	-	9.135	-
	E	F	E	F	E	F	E	F
Control	0.011	-	-	-	0.010	-	-	-
0.5	0.367	-	0.243	-	0.451	-	0.262	-
1.0	0.962	-	0.333	-	1.051	-	0.328	-
2.0	2.370	2.261	0.427	-	2.500	2.375	0.411	-
4.0	4.611	-	0.530	-	4.167	-	0.481	-
8.0	7.880	-	0.684	-	8.050	-	0.628	-
16.0	11.703	-	1.161	-	11.340	-	1.061	-

^aGrab samples were collected 24 and 96 hr after test began. Three diluters were used in duplicate: A and B, C and D, E and F.

^bThe part that passes through a 0.45-micron filter.

Table 38 lists the LC50 values obtained from the toxicity test started on May 8, 1972. One diluter failed to perform satisfactorily in these tests. The creek chub was the most sensitive species, and the striped shiner was the most resistant.

DISCUSSION

Water Quality Studies

The results of the water quality studies are discussed more fully in the general discussion (p. 168).

Copper was much less acutely toxic in Shayler Run water than would be predicted on the basis of hardness and alkalinity. Much of this reduced toxicity was related to copper detoxifying materials contributed by the effluent from the sewage treatment plant. Bioassays indicated that added phosphate detoxifies copper. The added phosphate from the sewage treatment plant could account for part of the reduced toxicity of copper in Shayler Run.

Even with this reduced toxicity, the acute toxicity of copper varied greatly with the changing water quality of Shayler Run. Acute bioassays have been and will continue to be an important tool for the establishment of water quality and effluent criteria. Our study indicates that varying toxicity due to varying water quality can be an important consideration in the establishment of water quality criteria for the protection of aquatic life.

Relative Sensitivity Studies

The wide range in sensitivity or resistance of different stream species to copper was an important consideration in the decision to use this metal as the toxicant in the Shayler Run study. The most striking result of the relative sensitivity studies was the high resistance of the bluegill to copper. The 96-hr LC50 (total measured copper) value for the bluegill was more than 10 times that of any other species tested in standard water. However, this variation of LC50 values was less than the variation of LC50 values of copper for both the bluntnose minnow and the fathead minnow due to water quality effects on copper toxicity. In the two tests in Shayler Run water in which the bluegill was used, it was the most resistant species.

In the bioassays conducted with standard water, the stoneroller, creek chub, rainbow darter, and bluntnose minnow were the most sensitive species, and their LC50 values were similar. The 96-hr LC50 values of these species were within the 95% confidence limits of each other. In the one toxicity test with Shayler Run water in which the stoneroller was used it was the most sensitive species. The relative sensitivity of the creek chub varied greatly in the two Shayler Run water tests. Except for the bluegill, the creek chub was the most resistant species in the bioassays conducted in May 1971; it was the most sensitive species in the May 1972 test.

In the standard water the bluntnose minnow was more sensitive than the fathead minnow. The 96-hr LC50 value for the bluntnose minnow was 0.34 mg/L. (0.29-0.40), and for the two fathead minnow bioassays it was 0.44 mg/L. (0.38-0.52) and 0.49 mg/L. (0.41-0.59). These LC50 values for the fathead minnow are similar to the value reported by Mount (1968) for the fathead minnow tested

TABLE 38. RELATIVE SENSITIVITY OF FIVE SPECIES OF FISH TO COPPER IN SHAYLER RUN WATER, ³ MAY 8, 1972

Species	Duplicate	Graphical interpolation				Probit analysis			
		LC50		LC50		LC50		LC50	
		Measured total copper (mg/l.)	Measured dissolved copper (mg/l.)	Measured total copper (mg/l.)	Measured dissolved copper (mg/l.)	Measured total copper (mg/l.)	Measured dissolved copper (mg/l.)	Measured total copper (mg/l.)	Measured dissolved copper (mg/l.)
		LC50	LC50	LC50	LC50	LC50	LC50	LC50	LC50
Brook trout	A	1.2	1.2	0.16	0.16	1.2	1.2	0.19	0.19
	B	1.1	1.1	0.14	0.14				
Rainbow darter		2.2	2.2	0.38	0.38	2.2	2.2	0.44	0.44
Fathead minnow ^a	A	1.2	1.2	0.09	0.09	1.2	1.2	0.11	0.11
	B	1.1	1.1	0.08	0.08				
Orange-spotted darter		1.2	1.2	0.08	0.08	1.2	1.2	0.11	0.11
Striped shiner	A	1.2	1.2	0.08	0.08	1.2	1.2	0.11	0.11
	B	1.1	1.1	0.08	0.08				

^a All fish water = hardness = 275 mg/l., as CaCO₃; alkalinity = 200 mg/l., as CaCO₃; pH = 8.3; test temperature = 17 °C.

^b Stat. analysis = probit 11.0 ± 1.1 (mg/l.) (unpublished).

in Newtown Laboratory water (hardness, 200 mg/L. as CaCO_3). He carried out three flow-through tests and obtained a 96-hr value of 0.47 mg/L. The LC50 values of copper for the bluntnose minnow in a test conducted in Shayler Run water were always lower than those for the fathead minnow. Most of the important species of fish in Shayler Run appeared to be at least as sensitive as the fathead minnow.

The 96-hr LC50 of the rainbow darter was about half that of the orangethroat darter in standard water. In four of the five tests in Shayler Run water, the rainbow darter was the more sensitive of the two species. In the bioassays with Shayler Run water, so much copper was precipitated on the bottom and in suspension above the bottom that it was impossible to see the darters that stayed on the bottom of the tank. The bottom of the tank had to be searched with a net to see if the fish were alive or dead.

Except for the bluegill, which was always the least sensitive species, the order of sensitivity of the species was not constant in the different dilution waters. Part of this change in order of sensitivity probably was due to the difficulty in the care and handling of some stream species.

The relationship of the LC50 values of the fathead minnow and the bluegill is a good example of the difficulty in comparing acute toxicity values of species tested in different dilution waters. Both of the species were easy to maintain under our laboratory conditions. In all cases the LC50 value of the bluegill was higher than that of the fathead minnow. However, because of water quality, the ratio between the values for these two species varied greatly. In the tests with standard water the LC50 (total measured copper) for the bluegill was about 20 times greater than that for the fathead minnow. In Shayler Run water (Tables 34 and 36) the LC50 value for the bluegill was only two times greater.

Another problem encountered in this comparative sensitivity study was the inability to distinguish and measure the toxic form or forms of copper. As a first approach to measure "toxic" copper, dissolved concentrations were measured. In standard water about 85% or more of the total copper was dissolved. As shown in Tables 35 and 37, at high concentrations only about 10% of the total measured concentration was dissolved. As the total copper concentration decreased, the proportion of dissolved copper increased until at the lowest concentrations the copper is about all "dissolved." As shown in both the flow-through and static bioassays, there is less variation in LC50 values for dissolved copper than in values for total copper. This suggests that insoluble copper is relatively nontoxic.

SECTION XIV

CHRONIC STUDIES

INTRODUCTION

To obtain laboratory test results for comparison with results from the field studies at Shayler Run, chronic toxicity tests with fish were conducted at both the Newtown Fish Toxicology Station (NFTS) and the streamside laboratory facilities adjacent to Shayler Run. At the NFTS laboratory before the field studies, three chronic tests with fathead minnows were conducted to determine the effect on reproduction of exposure to copper for different lengths of time before spawning. The three tests were performed at the same time and arranged to give 6 months, 3 months, and 0 months of exposure to copper before spawning. The tests were carried out because in the long-term stream exposure the different species of fish would be exposed to copper for different periods of time before spawning. The effect on reproduction was studied because Mount (1968) and Mount and Stephan (1969) had found that the MATC for the fathead minnow was established on the basis of egg production. Two flow-through acute toxicity tests were also conducted with the chronic tests so that an application factor could be calculated.

At the streamside laboratory, chronic tests with the fathead minnow and four resident stream species were conducted concurrently with the field studies. The tests were performed to obtain chronic toxicity data when the fish in the laboratory were under conditions as similar as possible to those in the stream so that a direct comparison of the results of the laboratory and the field exposure could be made. The tests used stream water from the control area and from the copper-treated area and were conducted under ambient conditions. A fry-growth and survival study was also conducted during the streamside studies with white sucker and creek chub fry.

METHODS

Newtown Fish Toxicology Station (NFTS) Chronic Tests

The methodology and design of the tests were similar to those described by Mount (1968). The three tests were conducted at the same time, November 1968 through October 1969. Copper was introduced immediately in the first test, 3 months later in the second, and immediately after the first spawning in the third, giving 6 months', 3 months', and 0 months' exposure to copper before spawning. The three exposure systems each consisted of a proportional diluter (Mount and Brungs, 1967) delivering 1 L. of control water and 1 L. each of six copper concentrations per cycle to duplicate exposure chambers. The exposure chambers were all glass and measured 30 by 60 by 30 cm high and contained 30 L. of water. The flow rate was about 7 tank-volumes of water a day. A dilution factor of

0.6 was used to give nominal concentrations of 100, 60, 36, 22, 13 $\mu\text{g/l}$. copper and control water. The concentrations were arranged randomly in each row of duplicate exposure chambers in one exposure system, and the same random arrangement was used in the other two systems.

The dilution water and the supply systems were the same as described by Mount (1968). Test temperature was controlled by a heater in the water-supply headbox and was modified by room temperature. Temperature was recorded by means of three 7-day indicating and recording thermographs, each with a probe in one chamber of an exposure system. For the first 4 months the average temperature was 23°C with a maximum of 26°C . During February the heater was off for 4 days, and the temperature averaged 15°C with a minimum of 11°C . There was an unanticipated early spawning on April 6. At that time the temperature was lowered to 19°C to approximate more closely the planned prespawning exposure periods to copper. The mean temperature slowly increased to 24°C by May and averaged 24.5°C during June, July, and August, when most of the spawning occurred. The mean temperature slowly dropped during September and reached 20°C at the end of the test.

Six pieces of half-tile were placed in each exposure chamber for spawning substrates. Light was provided by cool-white fluorescent ceiling fixtures controlled by two time switches. Early in December the day length was reduced from 16 to 10 hr. The day length was increased 1 hr every month until 16 hr were obtained in June, maintained for 2 months, and then decreased 1 hr every month.

All test fish were reared from eggs spawned in the laboratory by the fathead minnows obtained from the Newtown Fish Farm. Eggs were hatched, and fry were reared for about 4 weeks before they were randomly introduced into the exposure chambers. The fish were fed a commercial dry trout food, and live organisms in the water supply supplemented the diet. Excess food and waste products were siphoned from the exposure chambers as necessary. When a disease broke out, all 42 exposure chambers were given the same treatment. Potassium permanganate was used to control protozoan infections, and tetracycline and neomycin were used to control bacterial infections.

During the spawning season all tiles were examined in the early afternoon for eggs. Egg handling and hatching procedures were the same as described by Mount (1968). Hatchability was calculated as the percentage of larvae hatching from 50 eggs after 7 days of incubation.

Routine analyses of the test water from the exposure chambers were made in each set of duplicates every other week. Methods described by the American Public Health Association (1965, 1971) were used to measure oxygen in all seven chambers; hardness (EDTA) in the control chamber only; and pH, alkalinity, and acidity in two chambers. In addition, hardness was measured in the dilution water every weekday. The stock solution of copper for the toxicant-metering system was made as previously described by Mount (1968). During copper dosing, water samples for copper analysis were removed daily from each chamber and composited for 7 days. Each set of duplicate chambers was sampled on alternate weeks. After acidification, these samples were analyzed for total copper by means of the atomic absorption spectrophotometric procedure described in Section IX (p. 24).

Reproduction data from the three long-term exposures were subjected to a two-way analysis of variance using transformed values of $\sqrt{x} + \sqrt{x+1}$ where x is the mean number of eggs per female. The program used was "Analyses of Variance for Factorial Design" (Dixon, 1974).

Newtown Fish Toxicology Station (NFTS) Acute Tests

The acute toxicity of copper to fathead minnows from the same source as those used in the long-term test was determined with flow-through tests in NFTS water. The diluter was calibrated for a 0.75 dilution factor and delivered test concentrations to duplicate exposure chambers containing 10 fish each. Two tests were conducted: one with 6-week-old fish that averaged 22 mm in length, the other with 6-month-old fish that averaged 55 mm in length and 1.5 g in weight.

Streamside Chronic Tests

Procedures similar to those described for the NFTS tests were used for chronic tests with minnows and darters, and procedures similar to those described by Eaton (1970) were used for the chronic test with sunfish. Control water and water from the exposure area of the stream or control water dosed with copper in the laboratory were used for the tests. Five test systems were used for the streamside chronic tests over the 3-month study period; as many as three species were exposed in a test system. Roman numerals were used to designate the test systems. The numbers were assigned consecutively over the study period, I through V. Fathead minnows were exposed in test systems I, II, and IV; bluntnose minnows in test systems II, III, and IV; green sunfish in test system V; and johnny and fantail darters in test system III. Fish in test systems II through V were exposed to copper when copper was being metered to the stream. Fathead minnows in test system I were continuously exposed to a constant concentration of copper in stream water so a comparison could be made with fish intermittently exposed to copper in the other tests.

A proportional diluter (Mount and Brungs, 1967) was used in test system I to dose stream water from the control area with a stock solution of copper to obtain concentrations of 600, 350, 200, 120, 60, and 30 $\mu\text{g}/\text{L}$. copper and control stream water. In test systems II and III, modified proportional diluters were used to dilute the nominally dosed 120 $\mu\text{g}/\text{L}$. copper water from the exposure area with water from the control area to give nominal concentrations of 120, 60, and 30 $\mu\text{g}/\text{L}$. copper and control stream water. In test system IV a modified proportional diluter was used to give concentrations of 120 and 60 $\mu\text{g}/\text{L}$. copper and control water, and a toxicant-metering device, patterned after McAllister *et al.* (1972), was used to give a nominal 240 $\mu\text{g}/\text{L}$. copper concentration. For test system V a modified proportional diluter and metering device, as described for test system IV, was used when the test was started inside the laboratory during the winter. A proportional mixing of continuous flows of control water and exposure water and the continuous addition of copper solution from a Mariotte bottle and capillary-tube metering system to control water was used when the test was moved outside to give concentrations of 240, 120, and 60 $\mu\text{g}/\text{L}$. copper and control water. All test concentrations were set up in duplicate. Although toxicant concentrations were constant in test system I, they were not continuous or constant in test systems II through V since copper was not delivered to the test systems when copper was not being metered to the stream.

Glass tanks, 60 by 30 by 30 cm, were used for tests inside the laboratory with bluntnose and fathead minnows, and for the start of tests with bluntnose

minnows and darters in test system III. Plywood tanks were used for tests conducted outside and for the sunfish tests which required larger tanks. Plywood tanks, 120 by 60 by 30 cm, divided into 60- by 40-cm sections by plastic screens, were used to test the bluntnose minnow, the johnny darter, and the fantail darter in test system III. In test system V green sunfish were tested in 240- by 60- by 45-cm plywood tanks. In an attempt to lessen territorial fighting and loss of test fish, two partial dividers, 40- by 20- by 25-cm concrete blocks set on the long edge and extending across the tank from one side, were placed in each green sunfish tank. Smith (1975) suggests that there is considerable aggressive behavior and territorial fighting in green sunfish brought from the field into the laboratory. The sunfish tanks were also covered with nets, attached to one side of the tanks and weighted on the other side, to keep fish from jumping out or over to another tank. Water depth in the glass tanks was approximately 15 cm. Water depth in the 120- by 60- by 30-cm plywood tanks for test system III was 15 cm, and the depth in the sunfish tanks for test system V was 30 cm.

The flow of water for all of the tests was between 4 and 6 tank-volumes per day. When water flow to the tanks stopped because of clogged lines or pump failure, the tanks were aerated. During the first year and a half the air was turned on manually. After headboxes were installed, floats in the headboxes attached to microswitches turned on the air when the flow of water stopped. The test temperature followed ambient stream temperature $\pm 1^{\circ}$ C except in the bluntnose chronic test system III, in which electric immersion heaters in the headboxes and aquarium heaters in the tanks were used to maintain water temperature at about 24° C.

Spawning substrates for the minnows and darters and egg handling and hatching for the minnows were described by Mount (1968). Spawning substrates and egg hatching and handling for the sunfish were similar to those described by Eaton (1970). Fry growth chambers for sunfish were 30- by 15- by 30-cm glass tanks with screened ends placed in the sunfish tanks. Six spawning substrates were placed in each minnow and darter chamber, and three spawning substrates were placed in each sunfish tank.

The photoperiod for all tests, except for those in test systems I and IV, was the natural photoperiod for the season. Translucent green fiber-glass panels in the roof of the laboratory allowed passage of light from the outside. Cool-white fluorescent lights, turned on after sunrise and off before sunset by a timer, also provided light to test systems in the laboratory. Test system I was enclosed in opaque black plastic and had incandescent lights turned on by a timer to approximate the natural photoperiod pattern of southern Ohio. The bluntnose test in system IV, after 16 hr of daylight was reached naturally, was held at a 16-hr photoperiod by setting the timer for the laboratory lights to extend the spawning period for the bluntnose minnow.

Fish used for the chronic tests were seined or trapped from Shayler Run except for fathead minnows, which were obtained from the Newtown Fish Farm. Bluntnose minnows exposed in test system IV in one set of the nominal 120 $\mu\text{g/L}$ copper duplicates were from the exposure area of the stream. Green sunfish exposed in test system V in the two high nominal concentrations of 120 and 240 $\mu\text{g/L}$ copper were also from the exposure area. These fish were collected from the exposure area to determine the effect of prior and long-term exposure on their response in the tests. The green sunfish had been potentially exposed to copper in the exposure section of the stream for 2 years. The bluntnose

minnows had potentially been exposed to copper for 6 months before the test, and their parents had potentially been exposed to copper. The species, source, and age of fish used in the streamside chronic tests are summarized in Table 39.

The fish were held after collection from the field until sufficient numbers were available for testing. During holding and testing the fish were fed once a day ad libitum with dry trout food and frozen brine shrimp, or live *Daphnia* when available. The tanks were cleaned twice a week or more often if necessary. When external parasites and bacterial or fungal infections were observed, fish were treated. All tanks in a test system were treated similarly. Fungal infections were treated with 20 mg/l. Dexon®. A mixture of formalin (20 mg/l.) and Dexon® (20 mg/l.) was used for parasites, and a mixture of tetracycline (20 mg/l.), neomycin (12 mg/l.), and Dexon® (20 mg/l.) was used to treat bacterial infection. Dexon® was included for the treatment of parasitic and bacterial infection because secondary fungal infections usually were observed with disease or injury, or both. Problems with fungus were probably related to the high amount of organic material present in the water from the sewage treatment plant.

Dissolved oxygen, pH, alkalinity, hardness, and acidity were determined on grab samples of dilution water 5 days a week and on one of the duplicate control concentrations each week. Additional chemical analyses were made on other dilution water characteristics at less frequent intervals.

Water samples for copper analysis from the chronic tests were 7-day composited samples and were collected from one chamber of a set of duplicates on alternate weeks. Some grab samples were also taken from chronic tests in systems IV and V. Total copper and dissolved copper were determined weekly on chambers containing a nominal copper concentration of 120 µg/l.

Streamside Fry Growth and Survival

White sucker and creek chub fry were collected from Shayler Run and exposed in the streamside laboratory in flow-through systems to either control water or exposure water to determine the effects of copper on growth and survival. Two water-delivery systems (Brungs and Mount, 1970) delivered control or exposure water to duplicate sets of twelve 60- by 30- by 30-cm glass tanks. The water depth in the tanks was 15 cm, approximately 27 l. of water. The flow of water to the tanks was 6 tank-volumes per day. The drains of the tanks were covered with a fine-mesh stainless steel screen to prevent loss of fry.

The fry were captured in a soft nylon mesh dip net in the control area of the stream. They were sorted and identified, and fry of both species were randomized into five groups of 20-30 fry, depending on the number captured. Four of the groups were assigned to duplicate sets of control and exposure tanks. The remaining group was anaesthetized, and the lengths were measured to the nearest millimeter with a ruler. The average length calculated for that group was used as the initial length of the fry for the study. At 2 weeks, 4 weeks, and 8 weeks the fry were removed from the tanks, anaesthetized with MS 222, measured, and returned to the tanks. From the measurements an average length for each combined duplicate was obtained, and a t-test was used to determine if there was a significant difference between control and exposure groups of fry.

Temperature of the test water varied with the stream water temperature, and the photoperiod was the natural photoperiod for that time of year. The fish

TABLE 39. SOURCES OF FISH FOR STREAMSIDE CHRONIC TESTS

Test	Species	Source	Age-life stage	Size (mm)
Fry growth and survival	Creek chub	Control area of Shayler Run	Fry	14-21
	White sucker			12-13
Chronic tests				
System I	Fathead minnow	Newtown Fish Farm	5 months	32-38
System II	Fathead minnow	Newtown, Ohio	10 months	40-50
System III	Bluntnose minnow	Control area of Shayler Run	Early young-of-the-year	40-50
	Bluntnose minnow	Control area of Shayler Run	Early young-of-the-year	40-50
	Johnny darter		Young-of-the-year	30-40
	Fantail darter		Young-of-the-year	30-50
System IV	Fathead minnow	Newtown Fish Farm	Young-of-the-year	30-40
	Bluntnose minnow	Control area of Shayler Run	Late young-of-the-year	30-40
	Control 60 120 240 120 E			
System V	Green sunfish	Exposure area of Shayler Run	Early young-of-the-year	45-50
		Control area of Shayler Run	Adults	100-120
	Control 60 120 240			
		Exposure area of Shayler Run	Adults	100-120

were not fed during the study so that fry in the test would receive as nearly as possible the same food as fry in the stream. Loose material was siphoned from the tanks every week or after high stream flows when large amounts of suspended materials settled out.

RESULTS

Newtown Fish Toxicology Station (NFTS) Tests

The results of the routine chemical analyses of water from the exposure chambers are given in Table 40; the copper concentrations in the 7-day composited samples are given in Table 41. Survival of fathead minnows was not affected by continuous exposure to increasing concentrations of copper up to 100 $\mu\text{g/L}$. In the acute mortality tests there was 5% mortality at 160 $\mu\text{g/L}$ and 17.5% mortality at 280 $\mu\text{g/L}$. Survival of the fish in the long-term tests was related to disease, as fish died in several of the exposure chambers, but in no case was death related to copper concentration. The deaths that occurred after the number of animals was reduced by thinning were probably related to handling the fish.

Survival of the eggs that were spawned and incubated at the experimental concentrations was not adversely affected by copper (Table 42). In all three systems hatchability varied from 87% to 97%, all within the normal range of variation found at the NFTS. Thus, the length of time the adult fish were exposed to copper had no influence on hatchability of eggs.

The effect of copper on the fathead minnow reproduction is summarized in Tables 43, 44, and 45. During May at a photoperiod of 14 hr of light, spawning started in all systems. The first spawning in system III was on May 4, and copper was introduced on May 16 before the second spawning occurred. The last spawning was on September 22 at a photoperiod of 14 hr and mean temperature of 22° C. The test was terminated on October 23, 1969.

The reproduction data were examined by statistical analysis of variance for factorial design. The two controlled variables were length of time of copper exposure and copper concentration. The assumption was made that the measured concentrations at the same nominal concentrations for the three tests were not different. The statistical analysis indicated that the length of time of exposure to copper had no significant effect on the number of eggs per female. However, the effect of copper concentration was highly significant. To test which concentrations were different from the control, the data were analyzed with a one-sided Dunnett test. The analysis indicated that all nominal concentrations of 36 $\mu\text{g/L}$. (37 $\mu\text{g/L}$., measured) and higher caused a significant reduction in egg production ($P=0.05$). Nominal concentrations of 22 $\mu\text{g/L}$. (24 $\mu\text{g/L}$., measured) and lower had no significant effect. In summary, the statistical analysis indicated that the length of time the fish were exposed to copper had no effect on reproduction; that copper concentrations of 22 $\mu\text{g/L}$. (nominal) and lower had no effect on reproduction; and that copper concentrations of 36 $\mu\text{g/L}$. (nominal) and higher had a significant effect on reproduction.

Since the effect of pre-exposure to copper was not significant, the number of eggs per female for all six replicate chambers is summarized in Table 46. Egg production in the control and low copper concentration was less than that in 16 $\mu\text{g/L}$. However, there is a straight-line relationship between the mean number of eggs per female and the log of copper concentrations between 16 and 98 $\mu\text{g/L}$.

TABLE 40. WEEKLY CHEMICAL ANALYSES OF THE WATER IN THE EXPOSURE CHAMBERS FOR THE NFTS PRESPAWNING EXPOSURE CHRONIC TESTS WITH COPPER

Characteristic	Test 1 (6-month prespawning exposure)		Test 2 (3-month prespawning exposure)		Test 3 (0-month prespawning exposure)	
	Number of analyses	Mean $\pm$ S.D. ^b	Number of analyses	Mean $\pm$ S.D. ^b	Number of analyses	Mean $\pm$ S.D. ^b
Total hardness ^a (mg/l., as CaCO ₃)	18	204 $\pm$ 5.8	15	203 $\pm$ 5.1	14	204 $\pm$ 6.1
Alkalinity (mg/l., as CaCO ₃)	99	158 $\pm$ 8.1	96	158 $\pm$ 7.3	97	157 $\pm$ 7.0
Dissolved oxygen (mg/l.)	340	7.8 $\pm$ 0.79	337	7.8 $\pm$ 0.71	336	7.7 $\pm$ 0.72
Acidity (mg/l.)	91	8.0 $\pm$ 3.4	94	8.1 $\pm$ 3.0	93	8.7 $\pm$ 2.6
pH	98	7.9 ^c	95	7.9 ^c	98	7.8 ^c

^a Additional analyses of water leading to the test systems indicated: total hardness = 202 $\pm$ 6.1 (N=236); conductivity = 470 $\pm$ 50 (N=43).

^b S.D. = standard deviation.

^c Mode.

TABLE 41. TOTAL COPPER CONCENTRATIONS IN WEEKLY COMPOSITE SAMPLES FROM
THE NFTS PRESPAWNING EXPOSURE TOXICITY TESTS

Nominal copper concentration ($\mu\text{g/l.}$)	Duplicate	Test 1		Test 2		Test 3	
		(6-month prespawning exposure) Number of samples	Mean $\pm$ S.D. ($\mu\text{g/l.}$)	(3-month prespawning exposure) Number of samples	Mean $\pm$ S.D. ($\mu\text{g/l.}$)	(0-month prespawning exposure) Number of samples	Mean $\pm$ S.D. ($\mu\text{g/l.}$)
Control	A	24	4.9 $\pm$ 2.9	17	4.2 $\pm$ 1.1	10	3.5 $\pm$ 0.4
	B	24	4.2 $\pm$ 1.3	16	4.2 $\pm$ 1.5	10	4.3 $\pm$ 1.8
8	A	24	12 $\pm$ 1.6	17	11 $\pm$ 1.2	11	10 $\pm$ 1.3
	B	23	12 $\pm$ 1.5	17	11 $\pm$ 0.9	10	11 $\pm$ 1.2
13	A	24	16 $\pm$ 2.0	17	15 $\pm$ 1.4	11	16 $\pm$ 1.9
	B	23	17 $\pm$ 1.6	17	16 $\pm$ 1.4	10	16 $\pm$ 2.5
22	A	24	25 $\pm$ 2.3	17	22 $\pm$ 1.6	10	22 $\pm$ 2.6
	B	23	26 $\pm$ 3.1	17	24 $\pm$ 3.3	10	23 $\pm$ 2.3
36	A	24	38 $\pm$ 4.8	17	36 $\pm$ 2.8	10	38 $\pm$ 4.7
	B	24	38 $\pm$ 2.7	17	36 $\pm$ 1.7	10	38 $\pm$ 3.2
60	A	24	57 $\pm$ 7.4	17	57 $\pm$ 4.1	11	61 $\pm$ 6.4
	B	24	62 $\pm$ 4.5	17	56 $\pm$ 3.3	10	64 $\pm$ 5.8
100	A	23	97 $\pm$ 9.3	17	96 $\pm$ 7.8	11	98 $\pm$ 11
	B	23	101 $\pm$ 6.1	17	96 $\pm$ 10	10	101 $\pm$ 10

^aS.D. = standard deviation.

TABLE 42. HATCHABILITY OF EGGS FROM THE NFTS PRESPAWNING EXPOSURE CHRONIC TESTS

Nominal copper concentration ($\mu\text{g/L}$)	Duplicate	Test 1 (6-month prespawning exposure)			Test 2 (3-month prespawning exposure)			Test 3 (0-month prespawning exposure)		
		Number of eggs incubated	Number hatched	Percentage hatched	Number of eggs incubated	Number hatched	Percentage hatched	Number of eggs incubated	Number hatched	Percentage hatched
Control	A	500	456	91	500	450	90	460	419	91
	B	700	630	90	500	435	87	500	479	96
8	A	500	460	92	500	468	94	500	450	90
	B	550	520	95	500	470	94	500	459	92
13	A	500	468	94	500	465	93	400	375	94
	B	399	360	90	550	517	94	500	479	96
22	A	550	531	97	500	482	96	308	285	93
	B	450	422	94	350	334	95	500	434	87
36	A	500	474	95	400	371	93	497	437	88
	B	561	544	97	550	505	92	550	511	93
60	A	495	453	92	250	234	94	257	241	94
	B	250	224	90	450	407	90	485	439	91
100	A	0	0	-	202	196	97	200	177	89
	B	150	143	95	49	39	90	0	0	-

TABLE 43. NUMBER OF SPAWNS AND EGGS FROM FATHEAD MINNOWS WITH A
6-MONTH PRESPAWNING EXPOSURE TO COPPER

Nominal copper concentration ($\mu\text{g}/\text{l.}$)	Duplicate	Number of females	Number of males	Number of spawns	Number of spawns/ female	Total eggs produced	Number of eggs/ spawning	Number of eggs/ female
Control	A	10	6	49	4.9	5,700	116	570
	B	15	5	106	7.1	23,557	222	1,570
8	A	7	5	65	9.3	12,361	190	1,766
	B	8	4	60	7.5	10,052	168	1,257
13	A	11	5	100	9.1	15,881	159	1,444
	B	9	6	92	10.2	12,609	137	1,401
22	A	14	6	52	3.7	5,937	114	424
	B	17	4	102	6.0	13,453	132	791
36	A	13	5	45	3.5	5,189	115	399
	B	12	7	73	6.1	7,196	99	600
60	A	12	5	34	2.8	2,558	75	213
	B	11	8	8	0.7	708	88	64
100	A	15	4	1	0.1	11	11	1
	B	16	5	5	0.3	463	93	29

TABLE 44. NUMBER OF SPAWNS AND EGGS FROM FATHEAD MINNOWS WITH A 3-MONTH PRESPAWNING EXPOSURE TO COPPER

Nominal copper concentration (μg/l.)	Duplicate	Number of females	Number of males	Number of spawnings	Number of spawnings/female	Total eggs produced	Number of eggs/spawning	Number of eggs/female
Control	A	13	7	106	8.2	18,664	176	1,436
	B	13	7	33	2.5	5,538	168	428
8	A	8	6	30	3.8	3,318	111	415
	B	17	3	25	4.8	11,199	149	659
13	A	14	5	102	7.3	16,538	162	1,183
	B	9	10	59	6.6	8,281	140	920
22	A	8	6	35	8.8	6,255	179	1,364
	B	7	2	31	8.8	6,268	202	895
36	A	11	10	26	2.4	2,816	93	220
	B	13	7	56	4.3	6,676	119	513
60	A	14	6	10	0.7	713	22	51
	B	12	6	25	2.1	2,654	114	238
100	A	15	8	11	0.7	544	49	36
	B	11	9	2	0.2	70	35	6

TABLE 45. NUMBER OF SPAWNS AND EGGS FROM FATHEAD MINNOWS WITH
NO PRESPAWNING EXPOSURE TO COPPER

Nominal copper concentration ($\mu\text{g}/\text{l}$)	Duplicate	Number of females	Number of males	Number of spawnings	Number of spawnings/ female	Total eggs produced	Number of eggs/ spawning	Number of eggs/ female
Control	A	13	6	99	7.6	14,099	142	1,064
	B	13	6	63	4.8	8,930	142	687
8	A	11	9	54	4.9	6,703	124	670
	B	12	6	96	8.0	12,329	126	1,027
13	A	15	5	84	5.6	10,796	129	720
	B	13	6	87	6.7	13,142	151	1,011
22	A	11	8	29	2.6	3,855	133	390
	B	13	6	83	6.4	12,884	155	991
36	A	14	6	52	3.7	4,992	96	356
	B	12	7	86	7.2	8,798	102	733
60	A	12	8	11	0.9	843	76	70
	B	16	5	37	2.3	2,698	73	169
100	A	15	4	7	0.5	866	124	58
	B	12	8	0	0	0	0	0

TABLE 46. COMBINED EGG PRODUCTION BY FATHEAD MINNOWS IN THE SIX CHAMBERS FOR EACH CONCENTRATION OF COPPER

Copper concentration ($\mu\text{g}/\text{L.}$)		Total number			Eggs per female	
Nominal	Mean measured	Eggs	Males	Females	Mean number	Percentage of control
Control	4.2	76,508	37	77	894	100
8	11	55,964	33	63	888	99
13	16	77,267	37	71	1,088	122
22	24	48,652	35	66	836	94
36	37	35,787	42	68	526	59
60	60	10,674	38	77	139	16
100	98	1,954	34	84	23	3

The 96-hr LC50 values and 95% confidence limits were 0.49 (0.41-0.63) mg Cu/L. for 6-week-old fry and 0.46 (0.39-0.54) mg Cu/L. for the 6-month-old subadult fish, which indicates little effect of age on toxicity.

Streamside Tests

Analytical--

No measured dissolved oxygen concentrations were below 5 mg/L. in the test chambers in any chronic test. Hardness of the dilution water varied from 88 to 352, 110 to 356, and 84 to 330 mg/L., respectively, for the 3 months of the study. Alkalinity varied from 56 to 248, 66 to 256, and 50 to 236 mg/L., respectively, and pH from 7.5 to 8.5, 7.7 to 8.5, and 7.6 to 8.4, respectively. The chemical characteristics of the control test chambers are given with the results for each test. Some less frequently measured characteristics of the dilution water are reported in Appendix Tables 2 to 9. The total copper concentration for each of the chronic tests is reported in Tables 47 through 51. These values are the average measured total copper values of the composite samples of the test concentrations for the whole test period when the water was being dosed with copper. The average concentrations during the spawning periods of the combined composite samples for both duplicates are also listed. In winter and spring the copper concentration of the tests was lower since the stream was not dosed continuously because of high water. No attempt was made to adjust the values for average measured copper for samples lost, improperly taken or contaminated, or not measured. During the last 2 years of the study some of the weekly composite samples were discarded if the test was not dosed for more than 2 days for the week. The measured values for these samples probably would have been low, near the background value. Because most of the samples not measured probably would have been low, the average copper value given in the tables would be high. The fish in the test therefore would have been exposed to a slightly lower average value of copper than the measured average value indicated. The total numbers of samples not measured are reported in the tables. The average measured total values of copper will be used in the discussion of the results.

Fathead Minnow Chronic Test (Continuous Exposure)--Test System I--

The first fathead minnow chronic test was in test system I. Only fathead minnows were used. They were continuously exposed to nearly constant concentrations of copper for 9 months from January 13, 1970, to September 16, 1970 (Table 52). The temperature during the test ranged from 0° to 30° C. The hardness ranged from 148 to 340 mg/L., and the alkalinity ranged from 76 to 244 mg/L., with means of 274 and 183 mg/L., respectively. The pH ranged from 7.6 to 8.6. The average measured copper concentrations for the weekly composite samples were 561, 316, 180, 118, 66, 33, and 6.8 µg/L. (control) (Table 47). For 11 samples the mean dissolved copper in streamwater dosed with 118 mg/L. copper was 87% of the total (range 66 to 104). This test was similar to chronic tests with copper and fathead minnows conducted at the NFTS, in which the fish were exposed continuously to a constant concentration of copper except for the varying quality and temperature of the stream water.

Some fish died during the test because of disease. Deaths attributable to copper were observed in the three highest copper concentrations. These deaths were attributed to the effect of copper because there was no evidence of

TABLE 47. MEASURED TOTAL COPPER CONCENTRATION IN DUPLICATE TEST CHAMBERS OF TEST SYSTEM I - FATHEAD MINNOW CHRONIC TEST^a

Nominal copper concentration ($\mu\text{g/L}$)	Duplicate	Number of weekly composites analyzed	Mean measured copper concentration ($\mu\text{g/L}$)	Standard deviation	Range ($\mu\text{g/L}$)
Control	A B	14 14	7.2 6.5	2.7 1.7	5-14 4-11
30	A B	15 15	31 35	2 5	28-35 29-50
60	A B	16 15	64 67	6 7	55-76 58-88
120	A B	16 15	120 116	8 10	107-141 101-143
200	A B	16 15	180 181	14 16	156-208 135-201
350	A B	16 15	313 319	20 18	287-346 290-351
600	A B	16 15	557 565	33 25	487-610 525-617

^aData from Brungs et al. (1974).

TABLE 48. MEASURED TOTAL COPPER CONCENTRATIONS IN DUPLICATE TEST CHAMBERS OF
TEST SYSTEM II - FATHEAD AND BLUNTNOSE MINNOW CHRONIC TEST

Species	Nominal copper concentration ($\mu\text{g}/\text{l.}$)	Duplicate	Number of weekly composites analyzed	Mean measured copper concentration ($\mu\text{g}/\text{l.}$)	Standard deviation	Range ($\mu\text{g}/\text{l.}$)	Number of samples discarded
Fathead minnow	Control	A	2	9.5		7-12	1
		B	2	4.3		4-5	2
	30	A	3	29.0	8.7	19-35	0
		B	4	27.5	3.9	26-34	0
	60	A	3	49.3	12.7	35-59	0
		B	4	49.7	7.1	44-60	0
	120	A	3	86.3	23.7	60-106	0
		B	4	97.0	18.2	83-122	0
Bluntnose minnow	Control	A	2	5.4		5-6	1
		B	3	7.1	4.1	5-12	2
	30	A	3	28.3	6.7	21-34	0
		B	4	30.2	3.9	26-34	0
	60	A	3	51.3	14.4	35-62	0
		B	4	52.3	7.9	47-64	0
	120	A	3	85.7	27.2	55-107	0
		B	4	95.2	16.0	84-119	0

TABLE 49. MEASURED TOTAL COPPER CONCENTRATION IN DUPLICATE TEST CHAMBERS OF TEST SYSTEM III - BLUNTNOSE MINNOW CHRONIC

Nominal copper concentration (g/l.)	Duplicate	Test period				Spanning period				
		Number of weekly composites analyzed	Mean measured copper concentration (g/l.)	Standard deviation	Range (g/l.)	Number of samples discarded	Number of weekly composites analyzed	Mean measured copper concentration (g/l.)	Standard deviation	Range (g/l.)
Control	A	19	6.3	2.3	4-13	2	8	7.3	4.1	5-15
	B	19	6.5	2.5	4-15	2				
30	A	20	30.5	10.4	6-46	1	8	32.8	3.7	29-38
	B	20	30.0	10.1	7-46	1				
60	A	20	57.3	19.9	13-87	1	8	58.0	5.7	51-65
	B	20	53.4	19.4	10-85	1				
120	A	21	102.7	37.5	11-166	0	8	107	11.9	94-135
	B	21	98.1	35.2	14-166	0				

^aAverage for combined duplicates.

TABLE 50. MEASURED TOTAL COPPER CONCENTRATION IN DUPLICATE TEST CHAMBERS OF TEST SYSTEM IV - FATHEAD AND BLUNTNOSSE MINNOW CHRONIC TEST

Species	Nominal copper concentration (µg/l.)	Duplicate	Test period ^a				Spanning period ^b					
			Number of weekly composites analyzed	Mean measured copper concentration (µg/l.)	Standard deviation (µg/l.)	Range (µg/l.)	Number of samples discarded	Number of weekly composites analyzed	Mean measured copper concentration (µg/l.)	Standard deviation (µg/l.)	Range (µg/l.)	
Fathead minnow	Control	A	6	9.1	2.7	8-13	4	5	8.8	2.7	6-13	
		B	5	6.7	0.8	6-8	5					
	60	A	6	53	12.8	43-78	4	5	59.1	11.5	46-78	
		B	5	41	17.6	21-60	5					
	120	A	6	105	17.1	84-131	4	5	112.5	12.8	98-131	
		B	5	78	27.7	42-108	5					
Bluntnose	Control	A	10	10.3	5.5	5-24	6	See Table 57				
		B	9	9.1	3.1	5-14	6					
	60	A	11	43.1	11.3	17-57	6					
		B	9	45.1	9.1	30-57	6					
	120	A	9	84.8	12.3	67-104	7					
		B	8	91.6	12.4	41-115	7					
	120	AE	9	96.2	21.7	60-142	9					
		BE	6	87.0	31.1	42-111	9					
	240	A	16	190	53.7	90-279	5					
		B	14	195	46.2	97-243	6					

^aPeriod of 12/20/71 to 11/1/72 for both fathead and bluntnose minnows.

^bPeriod of 6/18/72 to 9/6/72 for the fathead minnow.

^cAverages for combined duplicates.

TABLE 51. MEASURED TOTAL COPPER CONCENTRATION IN DUPLICATE TEST CHAMBERS OF TEST SYSTEM V - GREEN SUNFISH CHRONIC TEST

Nominal copper concentration (µg/l.)	Duplicate	Fast period				Spawning period			
		Number of weekly composites analyzed	Mean measured copper concentration (µg/l.)	Standard deviation	Range (µg/l.)	Number of samples discarded	Number of weekly composites analyzed	Mean measured copper concentration (µg/l.)	Standard deviation (µg/l.)
Control	A	9	7.4	4.5	5.0-7.9	3	4	7.6	2.0
	B	7	9.0	2.8	6-14	5			
60	A	10	10.4	13.8	18-63	4	4	65.0	9.0
	B	7	41.6	13.2	29-62	5			
120	A	10	97.3	26.3	17-126	4	4	118.9	11.9
	B	7	84.4	10.6	47-124	5			
240	A	9	166	34.5	47-224	5	4	205.2	16.7
	B	7	162	36.6	10-216	5			

Average for combined duplicate test.

TABLE 52. SUMMARY OF EXPOSURE CONDITIONS FOR STREAMSIDE CHRONIC TESTS

Chronic test	Species	Number of fish per chamber	Date test began	Date spawning started	Date spawning ended	Date test terminated	Range of temperature during test (° C)	Range of temperature during spawning (° C)	Percentage of exposure time during test	Percentage of exposure time during spawning
System I	Fathead minnow	20	1-13-70	5-24-70	8-24-70	9-16-70	0-30	16-30	-	-
System II	Fathead minnow Bluntnose minnow	20	4-29-70	5-24-70	7-15-70	7-15-70	11-30	11-30	95	95
		20	4-29-70	5-11-70	7-15-70	7-15-70	11-30	11-30	95	95
System III	Bluntnose minnow Darters	25	9-24-70	6-1-71	8-6-71	9-3-71	0.5-30	17-30	80	93
		25	9-24-70	no spawning	no spawning	9-3-71	0.5-30	17-30	80	-
System IV	Fathead minnow Bluntnose minnow	20	12-22-71	6-18-72	9-6-72	10-1-72	0-29	14-29	60	84 ^b
		20	12-22-71	7-4-72	1-18-73	1-18-73	0-29	14-29	53 ^a	86 ^b
System V	Green sunfish	11-15	1-25-72	7-14-72	8-12-72	8-30-72	0-29	15-29	56	84

^aFrom 12-22-71 to 11-1-72, after 11-1-72 only the high concentration was dosed.^bFrom 7-4-72 to 11-1-72.

disease on other fish in the chamber from which the dead fish were removed and in other chambers of the test at that time. Within the first month of exposure, 38 of 40 fish died in the 561 $\mu\text{g}/\text{L}$. concentration, and 20 of 40 fish died in the 316 $\mu\text{g}/\text{L}$. concentration. By the end of the test 100% of the original fish placed in the 561 $\mu\text{g}/\text{L}$. copper concentration had died, and all but one of a second batch of fish placed in that concentration had also died. By the end of the test 75% of the fish at 316 $\mu\text{g}/\text{L}$. copper had died, and some deaths were attributable to copper in the 180 $\mu\text{g}/\text{L}$. copper concentration. No effect of copper on length or weight was found at the end of the test, possibly because of the small numbers of fish.

The first spawning in the test occurred on May 24, 1970, and the last spawning occurred on August 24, 1970. Exposure to copper resulted in complete blockage of spawning at concentrations of 561, 316, and 180 $\mu\text{g}/\text{L}$. (Table 53). Analysis of variance indicated that there was a significant difference at the 0.05 level in eggs per female among the different copper concentrations. A sequential variant of the Q-test (Hartley, 1955) indicated that at the 0.05 level the number of eggs per female was significantly different from the control at 118 $\mu\text{g}/\text{L}$. copper, but not at 66 $\mu\text{g}/\text{L}$.

Egg hatchability was not affected at any test concentration of copper even at 561 $\mu\text{g}/\text{L}$., where all adult fish died. In 6-10 batches of 50 eggs, better than 90% hatch was obtained in all concentrations of copper (6.8 (control)-118 $\mu\text{g}/\text{L}$.) in which eggs were produced. Duplicate batches of control eggs transferred to all other test concentrations had hatches of better than 90%.

In this test the most sensitive adverse effect was a decrease in number of eggs per female, and thus the MATC was between 66 and 118 μg copper/ L .

Fathead Minnow Chronic Test (for Development of Methods)--Test System II--

The second exposure of fathead minnows in the streamside laboratory was in test system II. Fathead minnows were exposed to copper for 2 1/2 months, April 29, 1970, to July 15, 1970. Copper was present in the test system 95% of the time during the test (Table 52). This test was primarily a methods-development test, because it was the first streamside test in which the copper-treated water from the exposure section of the stream was used for obtaining the test concentrations of copper. The nominal and measured copper concentrations are shown in Table 48. The fish used for this test were adults 10-11 months old, because younger fish would not have been mature enough to spawn. Temperature during this test ranged from 11° to 30° C (Table 52) and pH from 8.1 to 8.6. Hardness ranged from 230 to 346 mg/L . and alkalinity from 160 to 232 mg/L ., with means of 307 and 289 mg/L ., respectively.

There were no copper-related deaths in this test, although a few fish died as a result of disease in all concentrations. No effect on growth was determined because of the loss of the fish at the termination of the test before they could be weighed, measured, or sexed.

The first fathead minnow spawning occurred on May 5, 1970, after only 26 days of exposure to copper. The fish spawned in all test concentrations until the test was terminated. No effect of copper was apparent on total number of eggs, number of spawns, or eggs per spawn. The average measured high concentration for the test was 92 $\mu\text{g}/\text{L}$. (Table 54). Egg hatchability, based on

TABLE 53. SPawning AND EGG PRODUCTION BY FATHEAD MINNOWS IN CHRONIC TEST SYSTEM 1^a

Mean measured copper concentration ($\mu\text{g./l.}$)	Duplicate	Number of females during spawning	Number of males	Number of spawings	Spawings per female	Total eggs	Eggs per spawning	Eggs per female ^b
7.2 (control)	A	9	2	46	5.1	7,576	162	842
6.5 (control)	B	9	2	74	8.2	13,755	186	1,528
31	A	3.5	4	38	10.9	5,218	137	1,491
35	B	10	2	69	6.9	9,533	138	953
64	A	9	1	60	6.7	7,632	127	848
67	B	3.2	2	52	6.3	5,125	99	621
120	A	6	7	1	0.2	8	8	1
116	B	10	5	13	1.3	1,578	121	158
180	A	9	3	0	-	-	-	-
181	B	3	1	0	-	-	-	-
313	A	3	0	0	-	-	-	-
319	B	1	3	0	-	-	-	-
557	A	1	0	0	-	-	-	-
565	B	0	0	0	-	-	-	-

^aData from Brungs et al. (1974).

^bThese numbers have been adjusted to account for those females that died during the spawning season.

TABLE 54. SPawning AND EGG PRODUCTION BY FATHEAD AND BLUNTNOSE MINNOWS IN CHRONIC TEST SYSTEM II

Species	Nominal copper concentration ($\mu\text{g}/\text{L}$.)	Mean measured copper concentration ($\mu\text{g}/\text{L}$.)	Number of spawnings	Total eggs	Eggs per spawning
Fathead minnow	Control A	9.5	34	6,912	203
	Control B	4.3	30	5,072	169
	30 A	29.0	50	7,806	156
	30 B	27.5	12	2,538	211
	60 A	49.3	38	6,018	158
	60 B	49.7	51	9,051	177
	120 A	86.3	4	349	87
	120 B	97.0	38	4,113	108
Bluntnose minnow	Control A	5.4	21	2,212	105
	Control B	7.1	5	493	98
	30 A	28.3	8	536	67
	30 B	30.2	36	3,018	85
	60 A	51.3	37	4,289	115
	60 B	52.3	17	1,731	101
	120 A	85.7	15	1,231	82
	120 B	95.2	10	611	61

4-10 batches of 50 eggs per concentration, was high for all concentrations. Ninety-one per cent of all eggs hatched, and the lowest average hatch for any copper concentration was not less than 87%.

Fathead Minnow Chronic Test (Intermittent Exposure)--Test System IV--

The third exposure with fathead minnows was in test system IV. The test was started December 2, 1971, and ended October 1, 1972. The temperature during the test ranged from 0° to 29° C and the pH from 8.0 to 8.2. Hardness ranged from 156 to 300 mg/L., and alkalinity ranged from 120 to 230 mg/L., with means of 258 and 179 mg/L., respectively. The mean measured copper concentrations for the entire test were 91, 47, and 8.0 µg/L. (Table 50). The test fish were exposed to copper 60% of the time during the entire test and 84% of the time during spawning (Table 52).

No deaths attributable to copper occurred during the test, although some fish died early in the test because of disease. Copper had no effect on growth as measured by the size and weight of the fish at the end of the test.

The first spawning of the fathead minnow occurred on June 18, 1972 and the last on September 6, 1974. The fish spawned in all test concentrations. There was, however, a significant reduction at the 0.05 level in eggs per female at 112.5 µg/L. copper, the average concentration during spawning (Table 55). Based on spawning results, the safe concentration for the fathead minnow in this test would have been between 112.5 and 59.1 µg/L., the average measured copper concentration during spawning (Table 55).

Bluntnose Minnow Chronic Test (for Development of Methods)--Test System II--

Bluntnose minnows were exposed for the first time in test system II. This was a methods-development test, as discussed earlier for the fathead minnow in test system II. The exposure conditions and water chemistries for this test were the same as described for the fathead minnow test in system II.

There were no copper-related deaths in this test, although some fish died as a result of disease in all concentrations. No effect on growth could be measured because of the loss of the fish at the termination of the test before they were weighed, measured, or sexed.

The first spawning occurred on May 11, 1970, after only 13 days of exposure to copper. Fish spawned in all concentrations until the test was terminated. No effect was apparent on total eggs, number of spawns, or eggs per spawn. The average measured high concentration of copper for the test was 90 µg/L. (Table 54).

Egg hatchability based on 5-11 batches of eggs per concentration averaged 86% for all eggs hatched, and the lowest average hatch for any concentration was 82%. Success of hatch was not related to copper concentration.

Bluntnose Minnow Chronic Test (Intermittent Exposure)--Test System III--

The second exposure of bluntnose minnows to copper was in test system III, beginning September 24, 1970, and ending September 3, 1971. The test was conducted outside during the spawning period although the fish were initially

TABLE 55. SPawning AND EGG PRODUCTION BY FATHEAD MINNOWS IN CHRONIC TEST SYSTEM IV

Nominal copper concentration ($\mu\text{g}/\text{l.}$)	Mean measured copper concentration ($\mu\text{g}/\text{l.}$)	Mean ^a measured		Number of females during spawning	Number of spawning males	Number of spawning females	Spawning per female	Total eggs	Eggs per spawning female	Eggs per female
		copper concentration during spawning ($\mu\text{g}/\text{l.}$)	copper concentration ($\mu\text{g}/\text{l.}$)							
Control A	9.1	8.6		8	1	47	3.8	13,714	291	1,714
Control B	6.7			7	1	34	7.7	13,927	257	1,989
60 A	53	59.1		6	9	35	3.8	5,921	170	993
60 B	41			11	6	53	8.0	10,271	193	1,711
120 A	105	112.5		6	2	21	3.3	2,774	132	462
120 B	78			10	4	24	2.4	4,352	181	435

^a Average for combined duplicates.

exposed inside the streamside laboratory during the winter. The fish were young-of-the-year from the early (May-June 1970) spawn of the year. The fish were exposed to copper 80% of the time during this test (Table 52). The temperature range during the test was 0.5° to 30° C, and the pH range was 8.0 to 8.3. Hardness ranged from 220 to 324 mg/l., and alkalinity ranged from 156 to 240 mg/l., with means of 272 and 189 mg/l., respectively. The measured copper concentrations were 100, 55, 30, and 6.4 µg/l. (control) (Table 49).

Copper had no effect on growth or death of the test fish. Very few deaths occurred among the bluntnose minnows in this test compared to the number of fish deaths in the other chronic tests.

The fish spawned in all test concentrations of copper. The first spawn was June 1, 1971, and the final spawn was August 8, 1971. During spawning the fish were exposed to copper 93% of the time (Table 52), with an average high copper concentration of 107 µg/l. (Table 56). Based on spawning, the MATC was above 107 µg/l.

Bluntnose Minnow Chronic Test (Extended Spawning)-- Test System IV --

Bluntnose minnows were exposed for the third time in test system IV. The test started December 22, 1971, and ended January 18, 1973. During the test the temperature ranged from 0° to 29° C and the pH from 7.8 to 8.3. Hardness ranged from 156 to 328 mg/l. and alkalinity ranged from 102 to 230 mg/l., with means of 255 and 173 mg/l., respectively. To November 1, 1972, the average measured copper concentrations were 193, 91, 94, 44, and 10 µg/l. (Table 50), and the fish were exposed to copper 53% of the time during that period (Table 52). After November the copper concentrations of 94 µg/l. and below received only control water, while the 193 µg/l. concentration was dosed intermittently to determine if spawning could be blocked after it had started.

Deaths attributable to copper occurred among the bluntnose minnows in this test. All 20 fish in each duplicate, at the 193 µg/l. concentration, died within 70 days of the beginning of the test. Of the 15 additional fish added to each duplicate tank, two died in one tank at 193 µg/l. copper and three died in the other before the end of the test. These deaths also were apparently due to copper. The range of the measured copper values during the entire test was 78 to 279 µg/l. copper for weekly composite samples. A single grab sample on June 12, 1972, gave a value of 320 µg/l. copper.

Because the bluntnose minnows in most concentrations did not start spawning well during the normal spawning period, the test was extended after September by maintaining spawning temperatures and photoperiod (Table 52). On November 1, 1972, treatment of the stream with copper was stopped. Since the test system used copper-treated water from the exposure area of the stream to obtain concentrations of 94 µg/l. and below, exposure of fish at those concentrations was also stopped. Exposure of fish to 193 µg/l. was also stopped, although the toxicant-delivery system used for that concentration depended only on the flow of control water. Eleven days after copper exposure stopped, the bluntnose minnows in one tank of the 193 µg/l. concentration (218 µg/l. average copper concentration during the July 14 to November 1 spawning period) started to spawn, and 30 days later the fish in the other tank began to spawn. The spawning continued in both tanks until December 21 when copper was again introduced. Spawning stopped in the 193 µg/l. chambers the day after copper introduction and

TABLE 56. SPAWNING AND EGG PRODUCTION BY BLUNTNOSE MINNOWS IN CHRONIC TEST SYSTEM III

Species	Minimal copper concentration ($\mu\text{g/l.}$)	Mean ^a measured copper concentration		Number of males	Number of females during spawning	Number of spawnings	Spawnings per female	Total eggs	Eggs per spawning	Eggs per female
		Mean measured copper concentration ($\mu\text{g/l.}$)	Mean measured copper concentration during spawning ($\mu\text{g/l.}$)							
Bluntnose minnow	Control A	6.3	7.5	9	9	6	1.5	405	67	45
	Control B	6.5		7	16	22	1.3	1,283	58	80
	30 A	30.5	32.8	4	15	29	1.9	1,988	68	133
	30 B	30.0		5	19	26	1.4	2,111	81	111
	60 A	57.3	58	6	17	12	0.7	1,100	91	84
	60 B	53.4		11	10	28	2.8	1,801	64	180
	120 A	102.7	107	12	12	3	0.2	62	20	5
	120 B	98.1		6	17	26	1.3	1,834	70	107

^a Average for combined duplicates.

did not begin again before January 18, 1973, when the test was terminated (Table 57). The average measured copper concentration during the period, December 1 to January 18, was 130 $\mu\text{g}/\text{L}$., and the range was from 78 to 195 $\mu\text{g}/\text{L}$.. The fish in other concentrations, exposed only to untreated control water, continued to spawn. On the basis of spawning and survival, the safe concentration of copper in this test for bluntnose minnows was between 113 and 193 $\mu\text{g}/\text{L}$.. since the fish spawned at an average copper concentration of 113 $\mu\text{g}/\text{L}$., and spawning could apparently be blocked at 193 $\mu\text{g}/\text{L}$.. at which concentration death had also occurred early in the exposure.

In one tank at 91 $\mu\text{g}/\text{L}$.. copper concentration (113 $\mu\text{g}/\text{L}$.. average copper concentration during July 14-November 1 spawning period), the fish from the exposure area had been spawning since July 14, 1972 (Tables 50 and 57). Analysis of variance for the eggs-per-female data showed no significant differences among concentrations in any spawning period. Thus, the performance of the bluntnose minnows from the exposure area in the one set of duplicates at 91 $\mu\text{g}/\text{L}$.. copper was not statistically different from the fish from the control area used for the set of duplicates at 94 $\mu\text{g}/\text{L}$.. copper and other test concentrations. There was, however, poor duplication at all concentrations. The fish from the exposure area appeared to have spawned better. If they did spawn better, however, it was probably because they were larger when the test started, had matured earlier, and had started spawning earlier than the bluntnose minnows from the control area rather than because of any increase in resistance owing to previous exposure to copper or the exposure of their parents.

Green Sunfish Chronic Test--Test System V

Green sunfish were exposed to copper at the streamside laboratory only in test system V and in a preliminary spawning study. In the system V test they were exposed from January 25, 1972, to August 30, 1972 (Table 52). The temperature range during the test was 0-29° C, and the pH ranged from 8.0 to 8.3. Hardness ranged from 154 to 325 mg/L .. and alkalinity from 98 to 236 $\mu\text{g}/\text{L}$.. with means of 264 and 180 mg/L ., respectively. The measured average copper concentrations were 164, 91, 48, and 8.0 $\mu\text{g}/\text{L}$.. (Table 51). Copper was added only 56% of the time during the entire test period and 84% of the time during spawning.

No deaths were attributable to copper during the test, but many fish died as a result of secondary bacterial and fungus infection of wounds suffered during territorial fighting. All green sunfish in one control had died before spawning had started.

No conclusion on the effect of copper on growth of green sunfish was reached because of the limited number of fish left at the end of the test.

Spawning was poor in the 91 $\mu\text{g}/\text{L}$.. copper concentration, (119 $\mu\text{g}/\text{L}$.. average spawning concentration, Table 58, spawning probably was not related to copper concentration. Spawning in the 164 $\mu\text{g}/\text{L}$.. copper concentration (205 $\mu\text{g}/\text{L}$.. average spawning concentration) apparently was not different from the control or the 47 $\mu\text{g}/\text{L}$.. copper concentration. Since fish from the control area were used in the low concentrations of this test and fish from the exposure area in the high concentrations, the test was different from the other chronic tests. Apparently spawning was not affected in green sunfish that were exposed to copper in the exposure area of the stream for 2 years and were transferred to the same or a higher concentration.

TABLE 57. SPAWNING AND EGG PRODUCTION BY BLUNTNOSE MINNOWS IN CHRONIC TEST SYSTEM IV

Nominal copper concentration ($\mu\text{g/l.}$)	Percentage of time of exposure during test	Mean ^a measured copper concentration ($\mu\text{g/l.}$)	Number of males	Number of females	Number of spawnings	Spawnings per female	Total eggs	Eggs per spawning	Eggs per female
Spawning period 7-14-1972 to 11-1-1972 (copper)									
Control A	86	8.9	6	8	3	0.8	11	4	3
Control B			2	8	2	0.1	167	8	21
60 A	86	59	8	8	1	0.1	61	61	8
60 B			5	11	0	0	0	0	0
120 A	86	113	7	9	7	0.8	157	51	40
120 B			8	6	1	0.2	7	7	1
120 AE	86	113	1	6	8	1.3	110	64	85
120 BE			4	4	2	0.5	226	113	57
240 A	86	218	5	9	0	0	0	0	0
240 B			7	5	0	0	0	0	0
Spawning period 11-1-1972 to 12-21-1972 (no copper)									
Control A	0	NM ^b	6	4	0	0	0	0	0
Control B			2	8	6	0.8	94	90	68
60 A	0		6	8	0	0	0	0	0
60 B			5	11	6	0.5	593	83	18
120 A	0		2	9	4	0.5	61	15	7
120 B			8	6	2	0.3	32	16	9
120 AE	0		1	6	10	1.7	1,336	136	206
120 BE			4	4	1	0.3	31	31	8
240 A	0		4	4	11	1.2	1,041	95	116
240 B			7	5	7	1.4	800	43	60
Spawning period 12-21-1972 to 1-18-1973 (copper 240 $\mu\text{g/l.}$)									
Control A	0	9.7 ^c	6	4	1	0.8	193	69	49
Control B			2	8	1	0.4	334	111	12
60 A	0		8	8	1	0.4	32	11	4
60 B			1	11	7	0.5	26	105	53
120 A	0		7	9	1	0.1	22	22	2
120 B			8	6	6	1.0	192	65	65
120 AE	0		1	6	12	2.0	1,918	160	319
120 BE			4	4	5	1.3	452	90	111
240 A	80	130	4	9	0	0	0	0	0
240 B			7	5	0	0	0	0	0
Entire spawning period, 7-14-1972 to 1-18-1973									
Control A	53	10	6	4	6	1.3	206	34	51
Control B			2	8	11	1.4	1,042	95	131
60 A	53	30	8	8	8	0.5	93	24	12
60 B			5	11	13	1	1,207	91	92
120 A	53	60	7	9	12	1.3	440	36	49
120 B			8	6	9	1.3	531	50	75
120 AE	53	65	1	6	10	3.0	1,238	126	619
120 BE			4	4	8	2.0	709	89	118
240 A	53	126	4	9	11	1.2	1,041	95	116
240 B			7	5	7	1.8	800	43	60

^a Average value for combined duplicates.

^b Not measured - assumed to be background.

^c Value for combined duplicate controls A and B.

TABLE 58. SPawning AND EGG PRODUCTION BY GREEN SUNFISH IN CHRONIC TEST SYSTEM V

Nominal copper concentration ($\mu\text{g/l.}$)	Mean measured copper concentration ($\mu\text{g/l.}$)		Mean ^a measured copper concentration during spawning ($\mu\text{g/l.}$)		Number of males	Number of females during spawning	Number of spawning	Spawning per female	Total eggs	Eggs per spawning	Eggs per female
Control A	7.1		7.6		2	2	2	1	11,548	5,774	5,774
Control B			All fish died								
60 A	30.4		65.0		7	2	2	1	3,923	1,961	1,961
60 B	45.6				7	3	3	1	7,215	2,405	2,405
120 A	97.3		118.9		5	5	1	0.2	996	996	199
120 B	84.4				4	5	0	0	0	0	0
240 A	166		205.2		5	5	9	1.8	33,972	3,774	6,794
240 B	162				6	7	2	0.3	6,531	3,215	918

^a Average for combined duplicates.

In a preliminary spawning test with sunfish the previous year, longear sunfish and green sunfish were taken from the exposure area and exposed to copper at a nominal concentration of 120 $\mu\text{g}/\text{L}$ in test tanks in the laboratory for 2 weeks before spawning. The longear sunfish spawned an average of 1,377 and 1,315 eggs per female in duplicate chambers containing seven females in each chamber. In that test the green sunfish, treated in the same way as the longear sunfish, failed to spawn. The green sunfish were in 240- by 30- by 30-cm tanks in this preliminary test. Territorial disputes and overcrowding in the smaller tanks were assumed to be the reasons for the lack of green sunfish spawning in the preliminary study.

Egg hatchability, fry growth, and survival studies were attempted with the green sunfish eggs and fry from the chronic test in system V. However, low hatchability, because of fungus infection, and poor fry survival in all test concentrations precluded any conclusion relating to the effect of copper on these life stages.

Johnny and Fantail Darter Chronic Tests--Test System III--

The test conditions, copper concentrations, and water quality characteristics for johnny darters and fantail darters were the same as those described for the bluntnose minnow in test system III (Tables 49 and 52). The deaths that occurred in both species throughout the test were the result of disease and were unrelated to copper. Neither species spawned in any copper concentration, possibly because of overcrowding or other unacceptable test conditions. Analyses of fish lengths and weights at the end of the test showed no effect on growth related to copper concentration. Thus, copper had no effect on the growth and survival of the darters at a concentration of 107 $\mu\text{g}/\text{L}$.

Fry Growth and Survival in Streamside Tests--

The white sucker test was run for 2 months. After 4 weeks the same number of fry, 33, were alive in control and exposure tanks. The test was started with a total of 40 fry for each treatment. The average length of fry was 20.2 mm for the combined control duplicates and 16.4 mm for the combined exposure duplicates. The 3.8-mm difference was significant at the 0.05 level. After 8 weeks, survival was so low in both sets of tanks that the fish were not measured.

The creek chub test was run for only 4 weeks and terminated because of low survival in both control and exposure concentrations. At 2 weeks, however, all 40 fry were alive in both control and exposure concentrations, and there was a significant difference ($P=0.05$) in length. The length of fry was 20.7 mm for the combined control duplicates and 18.6 mm for the exposure duplicates.

DISCUSSION

Newtown Fish Toxicology Station (NFTS) Tests

The length of time that fathead minnows were exposed to copper before spawning had no influence on the effect of copper on egg production. Apparently the reduction in egg production due to copper is the result of exposure of the sexually mature fish during the spawning season, i.e., only the spawning season is the critical time of copper exposure.

The results of this chronic study were in excellent agreement with those reported by Mount (1968). In both studies the fathead minnow was exposed to copper in a standard (200 mg/l. hardness) water at the NFTS laboratory. Mount found that a copper concentration of 33 $\mu\text{g/l.}$ completely blocked spawning and that a copper concentration of 14.5 $\mu\text{g/l.}$ had no effect on spawning. In the present study a measured copper concentration of 37 $\mu\text{g/l.}$ significantly ($P=0.05$) reduced egg production, and the effect of a copper concentration of 12 $\mu\text{g/l.}$ was not significantly ($P=0.05$) different from the control. The intermediate concentration of 24 $\mu\text{g/l.}$ in this test also was not significantly ($P=0.05$) different from the controls.

The 96-hr LC50 values for the acute tests in this study were also in excellent agreement with the value reported by Mount (1968). The 96-hr LC50 value was 460 $\mu\text{g/l.}$ copper for subadult fish and 490 $\mu\text{g/l.}$ copper for 6-week-old fry. The mean 96-hr LC50 reported by Mount for three tests with adult minnows was 470 $\mu\text{g/l.}$ copper. Thus the application factor that lies between 0.05 and 0.08, based upon a MATC value between 37 and 24 $\mu\text{g/l.}$ and a continuous-flow 96-hr LC50 value of 460 $\mu\text{g/l.}$ found in this study, was in excellent agreement with the application factor reported by Mount (1968) that lies between 0.03 and 0.08, based upon a MATC value between 33.0 and 14.5 $\mu\text{g/l.}$ and a continuous-flow 96-hr LC50 value of 470 $\mu\text{g/l.}$

Although the "unsafe" copper concentration was similar in the two studies, Mount (1968) found no spawning in 33 $\mu\text{g/l.}$ whereas in the present study only 41% reduction in eggs per female in 37 $\mu\text{g/l.}$ was found. The difference may be due in part to the use of many more females in the present study. In chronic studies of this design, production of the individual female (except for zero eggs per female) cannot be quantified because only the mean of eggs per female per exposure chamber is determined. Mount (1968) exposed a total of eight females in the duplicate chambers, whereas 68 females were exposed in six exposure chambers in this study. Perhaps the large sample size was responsible for the graded effect, and the small samples size gave an all-or-none response.

Although the MATC value for copper was in agreement with the value reported by Mount, analyses of the results of the study were similar to Mount's because the data for all three exposures could be combined--six exposure chambers per concentration. If the analyses of variance had shown the results for the three exposures to copper to have been different, the data from each would have been analyzed separately. In that case the MATC values for the individual exposures would have been greater than the MATC found by Mount. Thus, with only a duplicate the ability of the test to detect statistically significant differences was lessened. The MATC values for the individual chronic tests were between 38 and 59 $\mu\text{g/l.}$ copper, 36 and 58 $\mu\text{g/l.}$ copper, and 38 and 62 $\mu\text{g/l.}$ copper for the 6-month, 3-month, and 0-month pre-spawning exposures, respectively. These values are about two times higher than the MATC value when the data for the three tests are combined.

Streamside Tests

The effects of copper on survival, growth, and reproduction of fathead and bluntnose minnows, green sunfish, and two species of darters as determined in the streamside chronic tests are summarized in Table 59. In general, copper had no effect on growth or egg hatchability. Effects on reproduction were the most sensitive indicator of toxic effects of copper studied in the chronic tests.

TABLE 59. SUMMARY OF STREAMSIDE CHRONIC TEST DATA

Chronic test system	Species	Mortality	Measured copper concentration ($\mu\text{g/l.}$)	Growth	Measured copper concentration ($\mu\text{g/l.}$)	Spawning	Measured copper concentration ($\mu\text{g/l.}$)	Eggs per female	Measured copper concentration ($\mu\text{g/l.}$)	Egg hatchability	Measured copper concentration ($\mu\text{g/l.}$)
I	Fathead minnow	100%	961	Insufficient data	Blocked	Reduced	118	No effect	561	No effect	561
		25%	316								
		Slight	181								
II	Fathead minnow	No effect	92	No data (specimens lost)	No effect	No data (specimens lost)	No data	No effect	92	No effect	92
	Bluntnose minnow	No effect	91	No data	No effect	No data	No data	No effect	91	No effect	91
III	Bluntnose minnow	No effect	100	No effect	100	No effect	107	No effect	107	Not done	Not done
	Darters	No effect	100	No effect	100	Did not spawn					
IV	Fathead minnow	No effect	91	No effect	100	No effect	113	Reduced	113	Not done	Not done
	Bluntnose minnow	100%	193	No effect	193	Blocked	218	No effect	113	Not done	Not done
V	Green sunfish	No effect	164	Insufficient data	No effect	No effect	205	No effect	205	Insufficient data	Insufficient data

Higher concentrations killed fish. A possible effect on fry growth at exposure-water concentrations of copper was indicated in the tests on fry growth and survival.

The statistical analysis of eggs-per-female data showed that in the fathead minnow chronic test in system I at 65 $\mu\text{g}/\text{L}$. copper, and in the fathead minnow chronic test in system IV at 59 $\mu\text{g}/\text{L}$. copper (the average copper concentration during spawning), egg production of the fathead minnows was not different from the control. At 118 $\mu\text{g}/\text{L}$. copper in the test in system I and at 112 $\mu\text{g}/\text{L}$. in the test in system IV, egg production was significantly different ($P=0.05$). The close agreement of these values indicates that there is little difference between the effect of continuous exposure to copper, as in test system I at an average concentration of 118 $\mu\text{g}/\text{L}$. copper, and the effect of intermittent exposure to copper at an average concentration of 112 $\mu\text{g}/\text{L}$. copper during spawning, as in test system IV. These results are also in general agreement with the laboratory fathead minnow study on the effects of different lengths of exposure to copper before spawning, which showed that the effect of copper is an acute effect on egg production during spawning. The fathead minnows in test system IV were exposed to copper less than 60% of the time before spawning.

Although results of streamside fathead minnow chronic tests in test systems I and IV indicate that the number of eggs per female was reduced between 66 and 118 $\mu\text{g}/\text{L}$. copper and 59 and 112 $\mu\text{g}/\text{L}$., respectively, the results of bluntnose minnow tests in systems III and IV indicate that reproduction of the bluntnose minnow was not reduced between copper concentrations of 58 and 107 $\mu\text{g}/\text{L}$. and 59 and 113 $\mu\text{g}/\text{L}$., respectively. These results contrast with the results of acute mortality tests with both stream and laboratory water, in which bluntnose minnows were more sensitive to copper than fathead minnows. It also contrasts with results of the chronic tests in which the extended mortality was greater for the bluntnose minnows. In the bluntnose minnow chronic test in system IV, at 193 $\mu\text{g}/\text{L}$. average copper concentration, all of the bluntnose minnows died within 70 days. In the fathead minnow test in system I, only a few fish died at 181 $\mu\text{g}/\text{L}$. copper. The results also disagree with those of a just-completed bluntnose minnow chronic toxicity test with copper in the NFTS laboratory that appears to show a graded response to increasing copper concentrations and an effect on eggs per female in the same range of copper concentrations as that found for the fathead minnow in the laboratory. The apparent reversal of the order of sensitivity in the streamside chronic tests in relation to the effect of copper on reproduction possibly results from a difference in the ability to detect a change in reproduction in the two species with the design of the test. A bluntnose minnow spawn averages about half the number of eggs in a fathead minnow spawn, and the number of eggs per spawn appears to be more variable. Thus a difference in number of eggs would be more difficult to detect in bluntnose minnows than in fathead minnows. Also, the spawning conditions in the streamside tests may not have been optimum for bluntnose minnow spawning.

In the streamside chronic tests green sunfish were more resistant than bluntnose and fathead minnows. Green sunfish spawned at an exposure concentration of 164 $\mu\text{g}/\text{L}$. copper and an average 205 $\mu\text{g}/\text{L}$. copper concentration during spawning. This order of sensitivity agrees with that found in acute mortality tests with sunfish and minnows in NFTS standard (200 mg/L . hardness) water and stream water.

In the streamside chronic tests apparently there was a seasonal difference in mortality related to water quality. Most of the deaths attributable to

copper in the fathead minnow chronic test in system I and the bluntnose minnow chronic test in system IV occurred near a copper concentration of 190 $\mu\text{g}/\text{L}$, or higher and during winter and early spring when acute static tests generally indicated increased copper toxicity. This may have been a result of the initial loss of the most sensitive individuals in the test population. However, in the bluntnose minnow chronic test in system IV, only 5 of the 30 bluntnose minnows in the second batch died when placed in the high concentration of copper, 193 $\mu\text{g}/\text{L}$, as compared to the death of all 40 in the initial batch. The presence of smaller amounts of detoxifying materials, which decrease the toxicity of copper, at this time would explain the difference in survival of the two batches of fish. No acute tests, however, were run concurrently with the bluntnose minnow chronic test in system IV.

The MATC values for copper in the streamside fathead minnow chronic tests systems I and IV were between 65 and 117 $\mu\text{g}/\text{L}$ copper and between 59 and 112 $\mu\text{g}/\text{L}$ copper, respectively. These MATC's were different from the MATC of between 24 and 37 $\mu\text{g}/\text{L}$ found in this study and the MATC of between 14.5 and 33 $\mu\text{g}/\text{L}$ copper found by Mount (1968) in constant-quality NPTS standard (200 mg/L hardness) test water and the MATC value of between 11 and 18 $\mu\text{g}/\text{L}$ copper in constant-quality (30 mg/L hardness) water determined by Mount and Stephan (1969). In fathead minnow streamside chronic tests in test systems I and IV, the average hardness was 274 and 272 mg/L , respectively. The change in hardness from 200 to 270 mg/L probably did not raise the MATC by a factor of four for the streamside tests. The higher values for the streamside chronic tests probably represented detoxification of copper by a complexing or precipitating agent in the stream water. As suggested by the affects of water quality on the acute tests, some detoxifying agent from the sewage treatment plant may have been responsible for the detoxification of the copper.

The average of the eight lowest total copper LC50 values (arbitrarily one-third of the total number of values) from the acute toxicity tests with fathead minnows in stream water was 1.2 mg/L copper. The application factor ranges, calculated from the 96-hr static LC50 value of 1.2 mg/L copper and the MATC values for fathead minnows in streamside chronic tests in test systems I and IV, were 0.05 to 0.10 and 0.05 to 0.09, respectively. These are in good agreement with the values found by Mount (1968) and those found in this study for constant-quality NPTS standard (200 mg/L hardness) water.

The application factor ranges of 0.05 to 0.10 and 0.05 to 0.09 for the continuous exposure of fathead minnows to copper in varying-quality water in test system I and for the intermittent exposure of fathead minnows to copper in varying-quality water in test system IV are also within the overall range of application factors of 0.02 to 0.24 found with fathead minnows, brook trout, and bluegills in continuous laboratory exposures in different constant-quality soft waters (Mount and Stephan, 1969; McKim and Benoit, 1971; Benoit, 1975).

PART D -- GENERAL DISCUSSION

SECTION XV

GENERAL DISCUSSION

The field study indicated that the fish in Shayler Run, except for the orangethroat darter, were adversely affected in some manner by the addition of 120 $\mu\text{g}/\text{l}$. of copper to the stream. Death, avoidance, and restricted spawning were the direct effects found. Fish deaths were observed only during a 1-week period beginning 7 days after initial introduction of copper, when a total of 62 fish (7 species) were observed dead. The number of fish killed was probably greater than the number recorded, however, because crayfish were observed eating dead fish. These early deaths were probably the result of sudden stress on the populations, which killed the most sensitive individuals.

Within the first 48 hr after initial addition of copper, bluntnose minnows and, to a lesser degree, stonerollers responded by moving downstream out of the exposure area. During the week after the initial copper introduction, streamside observations also indicated that an avoidance reaction to copper occurred with some species of fish. Large numbers, mainly striped shiners, stonerollers, bluntnose minnows, and a few sunfish and darters, were found congregating in areas where the copper concentration was less than 100 $\mu\text{g}/\text{l}$. as a result of the influx of spring or tributary water. As the stream warmed in the spring, bluntnose minnows, stonerollers, striped shiners, rainbow darters, and fantail darters began a mass exodus from the exposure area onto the downstream screens. This coincided with the onset of sexual maturation and spawning activity for the latter four species.

Copper adversely affected almost every common species of fish that spawned in the exposure or recovery areas by restricting the areas in which they could spawn. The single exception was the orangethroat darter, which spawned throughout both the control and exposure areas during the three seasons that copper was added to Shayler Run. During the exposure period bluntnose minnows spawned only in the lower exposure area where the total copper concentration averaged 60 $\mu\text{g}/\text{l}$. and ranged from 35 to 77 $\mu\text{g}/\text{l}$., even though they had spawned throughout the exposure area before the introduction of copper. Green sunfish and longear sunfish spawned throughout the exposure area during the spawning period before the introduction of copper, but in the succeeding 3 years these fish were limited to spawning areas where the copper concentration was 90 $\mu\text{g}/\text{l}$. or less. An additional indication of apparent reproductive impairment by copper was a sevenfold reduction in the number of fry in the exposure area.

The macroinvertebrate populations in the exposure area were generally reduced by copper. Four of the five most abundant forms, scuds, sowbugs, mayflies, and riffle beetles, were essentially eliminated. Chironomids were the one group that was not adversely affected; they flourished in the exposure area. Even though a dietary shift was evidenced by the green sunfish and the orangethroat darter, indirect effects on the fish populations through effects on the aquatic food chain could not be demonstrated.

Results of the field studies were consistent from year to year, considering the variations in stream flow, the varying amounts of time that copper was actually being added to the stream, and other unmeasured factors. Each year the fish populations generally declined, the macroinvertebrate populations were reduced or essentially eliminated, and the fish spawning was generally eliminated or reduced in the exposure area throughout the exposure period. Each of the various types of fish and macroinvertebrate collections complemented one another, showing essentially the same overall effect of copper on the biota of Shayler Run.

A wide range in sensitivity to copper was apparent in the different fish species. However, the relative order of sensitivity may have changed with changes in water quality or season. The bluegill was always the most resistant species. In general, the 96-hr LC50 values of most of the common stream species were lower than, but similar to that of the fathead minnow.

The acute bioassay results at both the Newtown Fish Toxicology Station (NFTS) and the streamside laboratories varied because of water quality variations of Shayler Run water. The 24-hr LC50 values varied from a high of 22 mg/L. to a low of 0.57 mg/L. for the bluntnose minnow. At the test temperature of 24° C, very few additional fish died after the first 24 hr. Even with the steady low-stream flow during October 1969 and the resulting steady water quality of alkalinity and hardness, the LC50 values varied from 22 to 0.75 mg/L. The 96-hr LC50 values of total copper for the fathead minnow varied from 23.6 to 0.92 mg/L.; the 7-day LC50 values varied from 23.6 to 0.56 mg/L. Thus the acute toxicity based on total copper in Shayler Run water varied about fortyfold for both species. In the initial toxicity tests with Shayler Run water, it was apparent that not only was there great variation in copper toxicity, but also that toxicity was less (LC50 values higher) than would be predicted on the basis of hardness, alkalinity, and pH of the stream water. This low toxicity was verified by more tests with the bluntnose and the fathead minnows. The mean 96-hr LC50 value of total copper to the fathead minnow was 8.9 mg/L. in Shayler Run water, with a mean hardness of 271 mg/L. and mean alkalinity of 183 mg/L. This mean value was much higher than the 96-hr LC50 value of 1.8 mg/L. reported by Pickering and Henderson (1965) in a dilution water that was higher in both hardness and alkalinity.

On the other hand, the variation of LC50 values of dissolved copper tested in Shayler Run water was much less than the variation of total copper values. For the bluntnose minnow, the dissolved copper 24-hr LC50 values varied only from 0.42 to 0.30 mg/L., whereas the 96-hr LC50 values for the fathead minnow, tested at ambient stream temperatures, varied from 1.4 to 0.52 mg/L. The constancy and smallness of these dissolved copper LC50 values, as compared to total copper values, indicate that insoluble copper, as measured, is relatively nontoxic. However, this does not mean that all copper that passes through a 0.45-micron filter is equally toxic since the copper associated with pyrophosphate that passes through the filter is not as toxic as other forms of copper that are present in the dissolved copper fraction.

It was suspected that the Shayler Run sewage treatment plant was discharging materials that were detoxifying copper in Shayler Run water. Bioassays, using diluent water from above and below the entrance of the effluent, indicated that copper was much less toxic in Shayler Run water below the plant. Additional toxicity tests, in which Shayler Run water was diluted with a reconstituted water similar in hardness and alkalinity, indicated that the reduction in toxicity was

not due to changes in hardness or alkalinity, but to some other detoxifying agent or agents being diluted.

Effluents from sewage treatment plants normally contain substantial concentrations of phosphates. Most of the total phosphorus measured in Shayler Run was orthophosphate. Orthophosphates react with copper to form an insoluble complex. Stepwise regression analyses indicated that there is a significant ($P=0.01$) correlation of total copper LC50 values and total phosphorous concentrations for both the fathead minnow and bluntnose minnow. Because this does not necessarily indicate a cause-effect relationship of high LC50 values and high phosphate concentrations, tests were conducted to determine the effect of added phosphates on the toxicity of copper. Results indicated that the added phosphates reduced the toxicity of total copper in both standard water and Shayler Run water. It is probable that the reduced toxicity of total copper in Shayler Run was due in part to the added phosphates from the sewage treatment plant.

Estimation of an unsafe level of copper ($120 \mu\text{g}/\text{L}$.) on spawning in the stream, by extrapolating from results of laboratory chronic tests with fathead minnows and acute tests with fathead minnows and stream species in constant-quality NFTS standard ($200 \text{ mg}/\text{L}$ hardness) water, was in agreement with results found for the minnow species in the stream. None of the minnow species spawned very successfully in the exposure area during the exposure period. Striped shiners and creek chubs were observed in prespawning activities in the exposure area, but no spawning was observed. A few bluntnose minnow eggs were observed in the extreme lower end of the exposure area. The average copper concentration during spawning seasons for the 3 years of exposure was approximately $106 \mu\text{g}/\text{L}$ in the upper exposure area at station 3 and $60 \mu\text{g}/\text{L}$ in the lower exposure area at station 6.

The results of the streamside bluntnose minnow laboratory chronic tests were not in close agreement with the predicted unsafe concentration or with what occurred in the stream. The bluntnose minnow in the streamside tests spawned in water from the exposure area as well as in the control water. Spawning of the bluntnose minnow was blocked in $218 \mu\text{g}/\text{L}$ copper in the extended chronic test in test system IV. This concentration was approximately two to three times the predicted unsafe level of copper and two to three times the concentration at which some bluntnose minnow spawns occurred in the exposure area. The reason for this apparent lack of agreement is not known. Considering however, that a 0.5 dilution factor was used for the test concentrations and that spawning was completely blocked at $218 \mu\text{g}/\text{L}$, there may have been a partial effect at some intermediate concentration which was not tested. The actual unsafe concentration in the chronic test may have been lower than $218 \mu\text{g}/\text{L}$ and nearer the $120 \mu\text{g}/\text{L}$, the predicted unsafe concentration. Additionally, other biological and physical factors in the stream that were not present in the streamside chronic tests may have had adverse effects on bluntnose minnow spawning.

The spawning observations on the green sunfish and longear sunfish in the stream suggest an effect of copper on spawning other than an effect on egg production. The green sunfish spawned at 205 $\mu\text{g/L}$ copper during the spawning period in the chronic test in test system V, and the longear sunfish spawned at 107 $\mu\text{g/L}$ in a preliminary test. In the stream they were not observed to spawn at copper concentrations above approximately 90 $\mu\text{g/L}$. The streamside laboratory tests and the predictions from the laboratory acute tests indicate that green sunfish could spawn at the highest concentration of copper in the exposure area. Since both sunfish species had spawned throughout the exposure area before the introduction of copper, the apparent restriction of the spawning area was possibly a behavioral response, which would not have been found or predicted from the chronic tests. Because there were effects in the stream not measured in the laboratory tests, these tests tended to underestimate the toxicity of copper in the stream. Based on extrapolation between species from a continuous to an intermittent exposure and from constant-quality to varying-quality water, the choice of 120 $\mu\text{g/L}$ was a relatively good estimate of the actual toxic copper concentration observed in the streamside laboratory chronic tests and in the stream.

The application-factor hypotheses as proposed by Nount and Stephan (1967) is a conceptual tool for estimating long-term safe conditions for fish. The application factor is defined as the maximum acceptable toxicant concentration (MATC) determined from a chronic toxicity test divided by the acute 96-hr LC50 value. They suggested that the application factor that is experimentally determined for a specific toxicant and species of fish might be applicable to the same toxicant and to other species of fish in other kinds of water.

This study on Shayler Run gave us an excellent opportunity to develop information concerning the usefulness of the application-factor concept as applied to a natural stream. The variable acute toxicity results found in Shayler Run water illustrate a practical problem in estimating safe concentrations of pollutants whose acute toxicity varies with water quality. The LC50 value to be used for estimating safe concentrations is important. Brungs *et al.* (1976) discuss the problems of calculating an application factor for copper from toxicity tests with the fathead minnow in a water of variable quality. They calculated an application factor of 0.04-0.07 based on low total copper LC50 values and an application factor of 0.07-0.13 based on dissolved copper measurements. They conclude that if dissolved metal concentrations were used to calculate mortality results and application factors, the predictive ability of the factors would improve.

The use of the application factor of copper for predicting safe concentrations for the bluntnose minnow in Shayler Run is also complicated by the variable water quality which causes varying acute toxicity values. For a series of toxicity tests, (Table 20) the LC50 values varied from 21 to 0.57 mg Cu/L, with a mean LC50 value of 7.9 mg Cu/L. Applying the application factor of 0.05-0.08 (found in our standard water test) to the mean total copper LC50 value would give an estimated safe concentration of 400-630 $\mu\text{g/L}$. The high concentration was found to be lethal during water quality conditions of low detoxifying conditions. Using the minimum total copper LC50 value of 0.57 mg/L, the estimated safe concentration in Shayler Run would be 29-47 $\mu\text{g Cu/L}$.

On the basis of the relative sensitivity tests, the estimated safe concentrations for many of the common stream species is similar to that calculated for the bluntnose minnow. The estimated safe concentration for the sunfish, however, would be much higher. Using the application factor of 0.07-0.13 based on dissolved copper measurements, the estimated safe concentration for the sunfish is 300-560 $\mu\text{g Cu/L}$. (dissolved) based on the mean dissolved copper 96-hr LC50 value of 4.3 mg Cu/L. for the bluegill.

Because of the effluent from the sewage treatment plant, Shayler Run probably has higher concentrations of materials that detoxify copper than would be found in most bodies of water. Thus, the variation in acute toxicity of copper probably is greater than would be found in many streams. The water quality studies emphasize, however, the importance of varying water quality in predicting safe concentrations for aquatic life.

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APPENDIX TABLE 1. SHAYLER RUN DISCHARGE AT GAGING WEIR, 1968-72^a
(IN CUBIC METERS PER SECOND)

Month	1968			1969			1970			1971			1972		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
Jan.				0.408	4.248	0.014	0.294	3.143	0.048	0.428	4.021	0.057	0.416	1.614	0.079
Feb.				0.210	1.076	0.079	0.498	2.549	0.085	1.354	6.768	0.051	0.600	2.265	0.068
March				0.153	1.218	0.048	0.799	3.568	0.082	0.657	2.917	0.142	0.847	3.908	0.198
April				0.552	2.152	0.093	1.985	18.887	0.085	0.118	0.425	0.051	1.065	5.134	0.165
May				0.317	1.982	0.042	0.239	1.444	0.028	0.280	1.897	0.051	0.685	3.341	0.057
June				0.037	0.105	0.014	0.051	0.187	0.017	0.074	0.190	0.023	0.180	2.039	0.028
July				0.033	0.140	0.011	0.057	0.453	0.018	0.045	0.178	0.020	0.041	0.113	0.022
Aug.	0.061	0.283	0.012	0.038	0.241	0.011	0.108	0.821	0.014	0.079	0.425	0.020	0.080	0.190	0.018
Sept.	0.014	0.031	0.010	0.026	0.105	0.010	0.032	0.139	0.018	0.524	2.152	0.034	0.124	1.189	0.015
Oct.	0.016	0.077	0.010	0.017	0.042	0.013	0.077	0.340	0.021	0.068	0.113	0.048			
Nov.	0.056	0.368	0.018	0.144	2.294	0.022	0.131	0.705	0.042	0.101	0.708	0.045			
Dec.	0.297	2.322	0.034	0.191	2.180	0.045	0.498	3.916	0.048	0.799	4.870	0.102			
Yearly				0.189	4.248	0.010	0.394	18.887	0.014	0.377	6.768	0.020			

^aPeak instantaneous discharge 67,961 m³/sec, April 2, 1970.

APPENDIX TABLE 2. MONTHLY TEMPERATURES FOR SHAYLER RUN, 1970-72
(IN DEGREES CENTIGRADE)

Month	1970			1971			1972		
	Mean	Maximum	Minimum	Mean	Maximum	Minimum	Mean	Maximum	Minimum
Jan.	1	2	0	1	5	0.5	6	13	1
Feb.	2	7	0	2	8	0	4	8	0
March	5	9	1	6	14	0	6	12	2
April	12	23	3	13	21	5	14	21	10
May	20	28	11	17	26	10	18	24	12
June	23	30	16	24	30	18	19	26	14
July	24	30	18	24	29	18	23	29	17
Aug.	23	28	19	22	27	17	23	28	18
Sept.	21	26	16	20	29	14	20	24	15
Oct.	13	19	8	15	24	10	14	20	7
Nov.	8	13	1	9	19	4	9	15	5
Dec.	5	13	0.5	7	14	3	7	11	4

APPENDIX TABLE 3. MONTHLY pH - IN SHAYLER RUN, 1970-73^a

Month	1970		1971		1972		1973	
	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum	Minimum	Maximum
Jan.	7.7	8.0	7.9	8.2	8.1	8.3	7.9	8.2
Feb.	7.7	8.0	7.9	8.2	7.9	8.2	7.8	8.1
March	7.5	8.0	7.7	8.1	7.9	8.3	8.0	8.4
April	7.6	8.3	7.9	8.1	7.9	8.3	8.0	8.3
May	8.0	8.4	7.8	8.3	8.0	8.3	8.0	8.8
June	8.0	8.4	8.0	8.4	8.0	8.4		
July	7.9	8.4	8.0	8.5	8.0	8.4		
Aug.	8.1	8.4	8.1	8.5	7.9	8.3		
Sept.	8.0	8.5	8.0	8.3	7.9	8.2		
Oct.	7.7	8.3	8.1	8.3	7.9	8.0		
Nov.	8.0	8.2	8.1	8.3	7.9	8.0		
Dec.	7.9	8.3	8.0	8.3	7.6	8.2		

^a Sampling occurred between 8:00 and 10:00 a.m.

APPENDIX TABLE 4. ALKALINITY OF SHAYLER RUN, 1970-73^a
(IN mg/l. AS CaCO₃)

Month	1970			1971			1972			1973		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Jan.	204	168	248	178	102	230	177	136	236	159	72	230
Feb.	151	78	204	157	90	248	170	100	230	149	71	194
March	142	92	192	150	66	196	154	98	188	150	93	199
April	155	56	198	206	172	224	150	68	210	152	141	171
May	188	96	220	193	146	222	165	110	226	199	175	240
June	212	160	228	192	164	216	207	172	228			
July	191	88	232	179	142	198	205	180	224			
Aug.	184	80	220	200	160	222	200	142	220			
Sept.	206	164	220	174	118	226	177	82	206			
Oct.	202	164	226	230	216	250	210	204	214			
Nov.	202	150	230	225	150	256	137	66	186			
Dec.	200	134	230	177	94	226	126	50	170			

^a Sampling occurred between 8:00 and 10:00 a.m.

APPENDIX TABLE 5. HARDNESS OF SHAYLER RUN, 1970-73^a
(IN mg/L. AS CaCO₃)

Month	1970			1971			1972			1973		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Jan.	309	264	350	253	168	316	255	200	326	219	118	282
Feb.	244	168	316	234	150	344	250	160	330	203	114	268
March	226	160	288	221	110	276	231	148	276	206	134	276
April	226	88	302	298	254	326	205	102	300	207	190	244
May	275	148	320	266	210	322	242	170	320	260	220	284
June	306	198	352	277	236	314	289	236	312			
July	278	124	336	271	204	300	301	270	312			
Aug.	253	112	310	287	214	316	307	222	324			
Sept.	310	236	350	236	186	310	272	144	330			
Oct.	292	230	342	310	300	328	305	298	310			
Nov.	284	210	316	316	224	356	178	106	240			
Dec.	286	212	320	240	148	300	180	84	236			

^a Sampling occurred between 8:00 and 10:00 a.m.

APPENDIX TABLE 6. DISSOLVED OXYGEN CONTENT OF SHAYLER RUN, 1970-73^a
(IN mg/l.)

Month	1970			1971			1972			1973		
	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum	Mean	Minimum	Maximum
Jan.	12.6	12.0	12.9	11.7	10.3	12.4	12.0	10.6	13.2	12.8	12.0	13.8
Feb.	12.4	10.5	13.0	11.7	10.8	12.2	12.6	11.9	13.4	12.0	10.6	14.4
March	11.3	8.0	12.9	10.9	9.4	13.0	11.2	9.5	13.1	11.2	9.1	13.3
April	10.2	8.6	12.1	8.7	7.3	10.3	10.4	9.6	11.1	12.2	10.1	14.2
May	8.8	7.0	10.2	7.8	6.6	9.3	9.2	8.1	10.6	8.2	6.0	9.7
June	7.0	6.0	7.4	6.4	5.9	8.0	7.6	6.7	9.4			
July	6.9	5.0	8.0	6.7	6.0	8.2	7.2	6.1	8.6			
Aug.	6.7	5.8	7.8	7.3	6.5	8.4	6.9	6.1	8.0			
Sept.	5.9	5.3	7.7	7.3	4.8	8.2	7.4	7.0	8.1			
Oct.	7.6	6.2	8.8	9.0	7.8	9.8	8.4	7.8	9.3			
Nov.	9.2	7.3	12.0	10.6	8.2	12.2	10.1	8.6	11.2			
Dec.	10.4	8.2	11.8	11.2	10.0	12.8	12.0	9.7	13.4			

^a Sampling occurred between 8:00 and 10:00 a.m.

APPENDIX TABLE 7. CHEMICAL CHARACTERISTICS OF SHAYLER RUN WATER BASED ON WEEKLY GRAB SAMPLES AND EXPRESSED AS MONTHLY MEANS, 1968-72

Year/Month	pH	Alkalinity mg/l. as CaCO ₃	Hardness mg/l. as CaCO ₃	SC umho/cm	TS mg/l.	DS mg/l.	Ca mg/l.	Mg mg/l.	OP-P mg/l.	TP mg/l.
1968										
Jan.	7.8	194	306	595						
Feb.	8.0	232	326	679						
March	7.8	127	200	469						
April	7.9	158	225	518						
May	7.8	175	250	550						
June	8.1	210	311	787						
July	7.8	193	278	840						
Aug.	7.8	220	289	842						
Sept.	7.8	220	328	1,060						
Oct.	7.6	196	301	956						
Nov.	7.8	186	272	743						
Dec.	7.6	158	268	532	378	366				
1969										
Jan.	7.7	238	328	580	457	446				
Feb.	7.8	162	237	468	361	346	95			
March	7.7	208	308	567	475	471	89	23		

APPENDIX TABLE 7 (continued). CHEMICAL CHARACTERISTICS OF SHAYLER RUN WATER BASED ON WEEKLY GRAB SAMPLES AND EXPRESSED AS MONTHLY MEANS, 1968-72

Year/Month	pH	Alkalinity mg/l. as CaCO ₃	Hardness mg/l. as CaCO ₃	SC umho/cm	TS mg/l.	DS mg/l.	Ca mg/l.	Mg mg/l.	OP-P mg/l.	TP mg/l.
1969										
April	7.7	174	250	502	364	356	81	19		
May	7.7	174	347	518	370	348	93	21		
June	7.8	218	302	781	505	505	93	21		
July	7.6	218	296	883	552	535	84	18		
Aug.	7.8	180	193	722	538	504	73	17		
Sept.				772	617	587	81	18		
Oct.	8.1	219	327	930	634	620	92	21		
Nov.	8.1	210	307	841	501	492	82	19		
Dec.	8.0	176	269	853	467	447	74	17		
1970										
Jan.	8.1	194	284	862	468	464	80	18		1.9
Feb.	7.8	148	243	678	384	383	67	14		1.0
March	8.0	139	212	530	340	277	56	12		0.6
April	8.3	163	214	526	431	322	70	13	1.1	1.5
May	8.1	186	264	613	411	401	77	14	1.9	1.9

APPENDIX TABLE 7 (continued). CHEMICAL CHARACTERISTICS OF SHAYLER RUN WATER BASED ON WEEKLY GRAB SAMPLES AND EXPRESSED AS MONTHLY MEANS, 1968-72

Year/Month	pH	Alkalinity		Hardness		SC umho/cm	TS mg/l.	DS mg/l.	Ca mg/l.	Mg mg/l.	OT-P mg/l.	TP mg/l.
		mg/l. as CaCO ₃	mg/l. as CaCO ₃	mg/l. as CaCO ₃	mg/l. as CaCO ₃							
1970												
June	8.1	206		304		872	570	560	89	18	3.8	4.0
July	8.1	197		280		795	496	481	84	18	4.3	4.4
Aug.	8.1	198		268		750	501	487	84	18	3.9	4.0
Sept.	8.0	205		308		858	593	577	93	20	5.4	5.4
Oct.	8.0	193		276		644	500	496	83	19	5.3	5.1
Nov.	8.1	191		257		602	406	397	78	18	2.3	2.5
Dec.	8.0	174		247		585	414	354	77	17	2.0	2.3
1971												
Jan.	8.1	195		272		632	402	396	81	18	1.2	1.6
Feb.	8.0	174		251		602	401	377	74	16	1.3	1.6
March	8.2	160		230		563	352	335	66	15	0.7	1.0
April	8.1	236		312		740	485	470	89	21	2.6	2.6
May	8.1	200		278		690	452	428	81	19	2.6	2.7
June	8.0	191		275		767	499	477	84	19	3.8	3.8
July	8.0	172		269		788	603	515	83	18	5.2	5.4

APPENDIX TABLE 7 (continued). CHEMICAL CHARACTERISTICS OF SHAYLER RUN WATER BASED ON WEEKLY GRAB SAMPLES AND EXPRESSED AS MONTHLY MEANS, 1968-72

Year/Month	pH	Alkalinity mg/l. as CaCO ₃	Hardness mg/l. as CaCO ₃	SC umho/cm	TS mg/l.	DS mg/l.	Ca mg/l.	Mg mg/l.	OP-P mg/l.	TP mg/l.
1971										
Aug.	8.2	191	279	725	545	496	82	18	4.2	4.2
Sept.	8.1	180	248	617	345	397	75	15	2.2	2.2
Oct.	8.1	227	306	797	480	468	89	20	3.3	3.3
Nov.	8.2	222	294	780	473	467	80	19	4.7	4.8
Dec.	8.2	177	240	553	354	340	66	15	0.6	0.7
1972										
Jan.	8.2	177	246	614	374	356	69	16	0.9	0.9
Feb.	8.2	162	243	623	396	366	75	16	0.9	1.0
March	8.4	166	241	612	356	339	69	16	0.5	0.6
April	8.1	142	198	477	442	280	53	12	0.6	0.6
May	8.1	194	262	616	397	367	70	16	0.9	1.0
June	8.1	215	275	594	479	461	92	19	2.4	2.4
July	8.1	198	298	813	556	517	87	19	3.4	3.4
Aug.	8.1	192	310	1,009	651	620	84	20	5.1	5.3
Sept.	8.0	181	280	866	587	558	74	19	8.9	9.0
Oct.	8.1	207	300	813	537	527	87	19	6.2	6.2

APPENDIX TABLE 7 (continued). CHEMICAL CHARACTERISTICS OF SHAYLER RUN WATER BASED ON WEEKLY GRAB SAMPLES AND EXPRESSED AS MONTHLY MEANS, 1968-72

Year/Month	pH	Alkalinity		Hardness		SC	TS	DS	Ca	Mg	OP-P	TP
		mg/l. as CaCO ₃	mg/l. as CaCO ₃	mg/l. as CaCO ₃	mg/l. as CaCO ₃							
1972												
Nov.	8.1	126		185		446	292	272	56	11	0.4	0.6
Dec.	8.1	137		188		350	338	263	57	12	0.6	0.8

APPENDIX TABLE 8. CHEMICAL CHARACTERISTICS FOR SHAYLER RUN BASED ON WEEKLY GRAB SAMPLES AND EXPRESSED AS MONTHLY MEANS, 1970-73

Year/Month	Na mg/l.	K mg/l.	CHD mg/l.	NO ₃ -N mg/l.	NO ₂ -N mg/l.	NH ₃ -N mg/l.	ORG-N mg/l.	TKN mg/l.	TP mg/l.	TOC mg/l.	Ca mg/l.	Hg mg/l.
1970												
Jan.	46	5.0	81	1.4	0.3	2.2	0.8		1.9	6.6		
Feb.	34	4.4	61	1.5	0.1	0.4	0.7		1.0	5.7		
March	21	4.2	38	1.2	<0.1	0.4	0.8		0.6	8.0		
April	21	3.8	31	0.5	<0.1	0.9	0.4		1.0	5.5		
May	26	4.0	34	1.6	<0.1	0.5	0.4		1.8	6.0		
June	54	8.2	66	4.1	<0.1	<0.1	0.6		3.4	5.6		
July	51	10.7	64	2.9	<0.1	<0.1	0.4		3.9	6.2		
Aug.	48	7.8	54	3.4	<0.1	0.2	0.3		3.8	4.3		
Sept.	65	9.0	76	7.5	<0.1	0.1	0.9	0.5	5.0	6.0		
Oct.	54	8.4	60	5.5	<0.1	1.4	0.4	1.4	4.6	7.3		
Nov.	24	4.6	43	2.0	0.2	1.8	<0.1	1.9	2.4	7.0		
Dec.	20	4.3	39	2.5	0.1	1.6	0.3	1.7	1.9	7.3		
1971												
Jan.	24	5.1	37	1.4	0.1	2.6	0.5	2.6	1.6	5.4		
Feb.	23	3.8	41	1.1	<0.1	1.9	0.6	2.5	1.1	7.4		
March	24	3.5	34	0.6	0.1	0.9	0.5	1.3	1.1	5.1		
April	36	5.1	50	3.0	<0.1	0.4	0.4	0.9	3.0	5.7		

APPENDIX TABLE 8 (continued). CHEMICAL CHARACTERISTICS FOR SHAYLER RUN BASED ON WEEKLY GRAB SAMPLES AND EXPRESSED AS MONTHLY MEANS, 1970-73

Year/Month	Na mg/l.	K mg/l.	CHD mg/l.	NO ₃ -N mg/l.	NO ₂ -N mg/l.	NH ₃ -N mg/l.	ORG-N mg/l.	TKN mg/l.	TP mg/l.	TOC mg/l.	Ca mg/l.	Mg mg/l.
1971												
May	34	5.2	49	1.9	0.1	0.4	0.3	0.9	2.9	5.9		
June	44	6.2	56	4.1	0.1	0.2	0.8	1.0	3.6	5.7		
July	51	7.9	62	7.2	<0.1	0.2	1.8	1.9	4.9	11.3		
Aug.	50	7.9	62	4.2	<0.1	0.5	0.9	1.4	4.4	7.6	82	18
Sept.	32	5.2	39	1.6	<0.1	<0.1	0.7	0.7	2.2	6.1	75	16
Oct.	44	6.2	46	2.3	<0.1	0.1	0.3	0.4	3.1	7.0	90	20
Nov.	39	6.3	46	2.8	<0.1	0.4	0.4	0.8	3.8	7.0	89	20
Dec.	19	3.9	27	1.4	<0.1	0.3	0.4	0.7	0.9	7.3	71	16
1972												
Jan.	23	3.8	33	0.8	<0.1	0.4	0.1	0.5	1.0	5.7	73	16
Feb.	28	3.6	46	1.8	<0.1	0.5	0.8	1.3	1.1	9.1	75	16
March	20	2.2	37	1.2	<0.1	0.1	0.2	0.5	0.6	6.5	69	16
April	16	2.6	24	0.8	0.2	0.1	2.8	2.2	0.7	13.4	53	12
May	24	5.0	28	1.5	<0.1	0.2	0.4	0.6	1.2	7.5	70	16
June	34	5.0	47	3.4	0.1	0.3	0.6	0.9	3.0	8.6	92	19
July	46	7.6	60	6.0	0.1	0.1	0.7	0.8	4.2	8.3	87	19
Aug.	60	10.9	82	12.0	<0.1	0.4	0.8	1.1	6.8	9.1	84	20
Sept.	49	10.8	67	6.8	<0.1	0.1	1.0	1.1	6.5	8.4	74	19

APPENDIX TABLE 8 (continued). CHEMICAL CHARACTERISTICS FOR SHAYLER RUN BASED ON WEEKLY GRAB SAMPLES AND EXPRESSED AS MONTHLY MEANS, 1970-73

Year/Month	Na mg/l.	K mg/l.	CHD mg/l.	NO ₃ -N mg/l.	NO ₂ -N mg/l.	NH ₃ -N mg/l.	ORG-N mg/l.	TKN mg/l.	TP mg/l.	TOC mg/l.	Ca mg/l.	Mg mg/l.
1972												
Oct.	47	8.4	56	5.4	0.1	0.6	1.0	1.6	5.3	7.5	87	19
Nov.	15	2.8	22	1.3	<0.1	0.1	0.8	0.9	0.7	7.1	56	11
Dec.	15	2.9	22	2.9	<0.1	0.5	0.6	1.1	0.7	6.9	57	12
1973												
Jan.	20	2.2	26	1.2	<0.1	0.9	0.5	1.0	1.0	5.8	56	15
Feb.	20	3.2	30	1.0	0.1	1.1	<0.1	0.9	0.9	5.2	65	15
March	19	2.0	30	1.2	<0.1	0.4	0.3	0.7	0.5	5.1	58	13

APPENDIX TABLE 9. METAL CONCENTRATIONS IN SHAYLER RUN, 1968-72
(AS $\mu\text{g/l.}$)

Metal	5-17-68	10-30-68	2-18-69	7-2-69	10-21-69	5-14-70	11-3-70	12-2-70
Zn	<21	46	<20	<23	125	63	<23	<15
Cd	<21	<19	<20	<23	<36	<17	<23	<15
Ar	<107	<50	<100	<50	<50	<50	<115	<75
B	205	830	260	490	1,360	205	445	340
P	610	3,700	940	1,200	7,150	<85	910	705
Fe	<11	9	130	240	820	1,165	120	215
Mo	<43	61	110	55	<71	<33	<45	<30
Mn	<11	<9	48	70	<18	37	28	24
Al	<43	<37	190	245	750	1,230	50	205
Be	<0.21	<0.19	1.0	<0.23	<0.36	<0.17	<0.23	<0.15
Cu	<11	27	13	<11	18	25	<11	<8
Ag	<2.1	<1.9	<2.0	<2.3	<3.6	<1.7	<2.3	<8
Ni	<71	<19	<20	<23	<36	7	<23	<15
Co	<21	<19	30	<23	<36	3	<23	<30
Pb	<43	<37	60	<45	<71	<50	<45	<30
Cr	<11	<9	19	<11	<18	<8	<11	<8
V	<43	<37	<40	<45	<71	<33	<45	<30
Ba	33	30	33	38	25	36	24	20
Sr	190	380	184	320	170	155	45	165

APPENDIX TABLE 9 (continued). METAL CONCENTRATIONS IN SHAYLER RUN, 1968-72
(AS $\mu\text{g/l.}$)

Metal	3-12-71	5-19-71	8-5-71	11-3-71	4-27-72	5-12-72	6-1-72
Zn	<13	<22	<30	<30	<20	<20	<20
Cd	<13	<22	<15	<15	<20	<20	<20
Ar	<63	<50	<80	<80	<80	<80	<80
B	25	300	340	700	260	100	280
P	<63	585	700	1,600			
Fe	590	17	600	50	400	80	120
Mo	<25	<43	150	140	<20	20	<20
Mn	15	<11	50	<8	400	<20	<20
Al	620	<43	760	80	>1,000	>1,000	>1,000
Be	<0.13	<0.22	<0.2	<0.2	<0.1	<0.1	<0.1
Cu	<6	<11	15	8	<20	<20	<20
Ag	<1.3	<2.2	<2	<2	<2	<2	<2
Ni	<13	<22	<15	<15	<20	<20	<20
Co	<13	<22	<15	<15	<20	<20	<20
Pb	<25	<43	60	<50	<40	<40	<40
Cr	<6	<11	<15	<15	<20	<20	<20
V	<25	<43	<15	<15	<40	<40	<40
Ba	16	42	15	15	24	20	20
Sr	81	160	200	260			